CONTRIBUTIONS IN MARINE SCIENCE

VOLUME 12 | JULY, 1967

CONTRIBUTIONS IN MARINE SCIENCE

VOLUME 12, JULY 1967

PUBLISHED BY

THE UNIVERSITY OF TEXAS
MARINE SCIENCE INSTITUTE

at

PORT ARANSAS, TEXAS

CONTRIBUTIONS IN MARINE SCIENCE

DIRECTOR'S NOTE

Beginning with this issue, Volume 12, the former **Publications of the Institute of Marine Science** becomes **Contributions in Marine Science** with a new cover and format. Two considerations make this change timely.

Following action by the University of Texas Board of Regents, pursuant to provisions of Senate Bill 14, 60th Texas Legislature, the Institute of Marine Science of The University of Texas is now The University of Texas Marine Science Institute at Port Aransas.

In recent years, a considerable number of research papers by authors from many institutions appeared along with those by staff from The University of Texas. The use of the old designation, *Publications of the Institute of Marine Science*, was thus inaccurate.

Because of the official name change of the Institute and because of the desire to continue publication of scholarly contributions that have a general pertinence to marine science, the new name *Contributions in Marine Science*, is more accurate and appropriate.

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Original papers based on research may be submitted regardless of whether or not the author is or has been connected with the University of Texas.

Manuscripts (in triplicate) should be sent to the Editor, The University of Texas Marine Science Institute at Port Aransas, Texas, 78373.

Papers are accepted for consideration any time from July 1 of previous year to February 1 of the year of publication; all manuscripts submitted after February 1 will be considered for the succeeding volume.

All papers are reviewed by three referees.

In most respects the *Style Manual for Biological Journals* of the American Institute of Biological Sciences is used (2000 P St. NW, Washington, D.C. 20007). Bibliographic abbreviations follow *World List of Scientific Periodicals* (Butterworths).

All manuscripts are to be in English, although the abstract may be presented in another "congress" language in addition to English. Metric and Celsius units are to be used instead of or in addition to English and Fahrenheit units.

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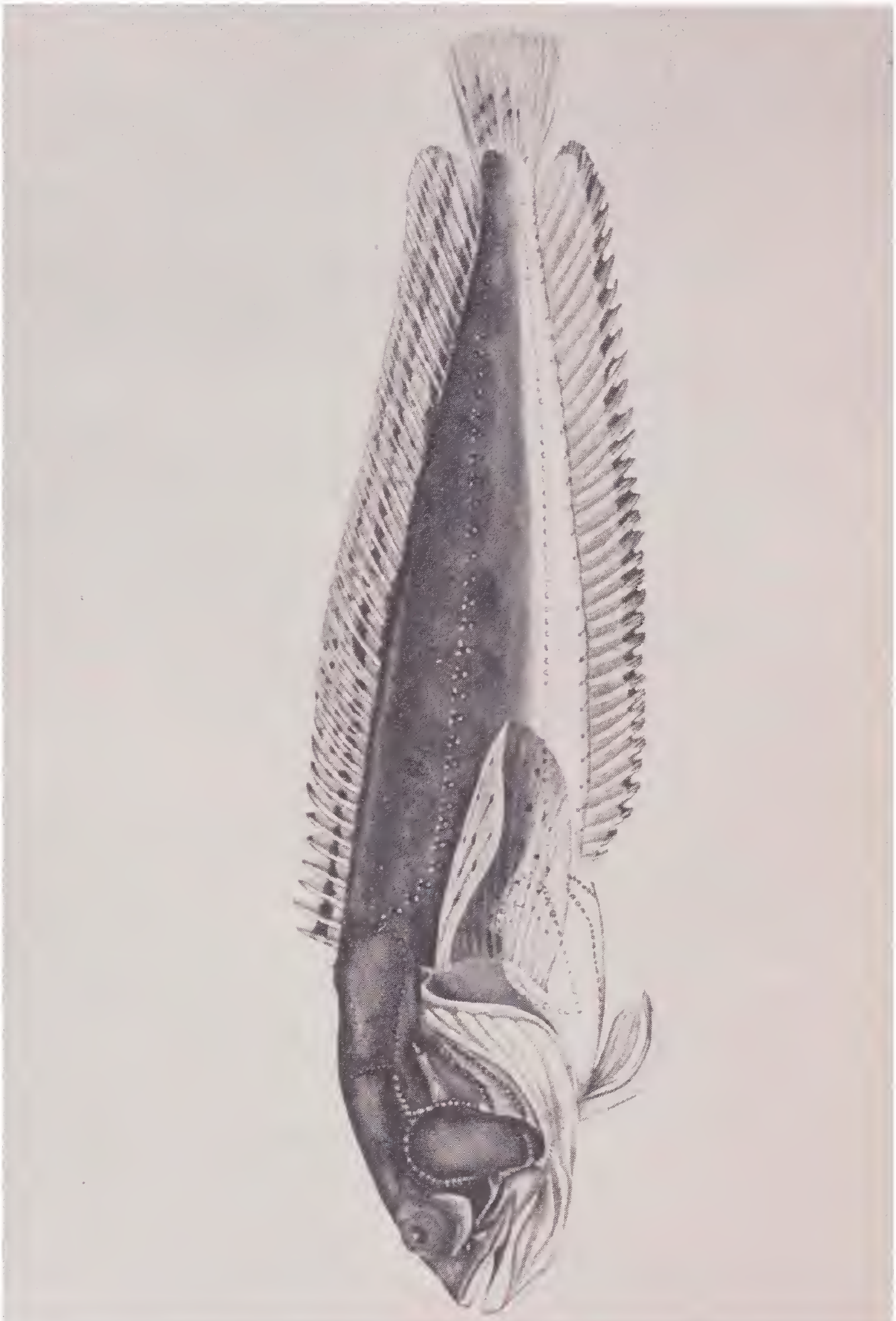

A STUDY OF THE ATLANTIC MIDSHIPMEN, *PORICHTHYS POROSISSIMUS*, IN THE VICINITY OF PORT ARANSAS, TEXAS[1]

E. D. Lane[2]

The University of Texas Marine Science Institute at Port Aransas, Texas

ABSTRACT

Life history information is presented for the Atlantic midshipman (*Porichthys porosissimus* [Valenciennes]; Batrachoididae). Most data were obtained from fish caught by trawling in the bays and the shallow Gulf of Mexico around Port Aransas. Atlantic midshipmen occur from Virginia to Argentina and exhibit variation in color pattern and meristics throughout this range. They also show microgeographical meristic variation between the bays and the Gulf on the Texas coast. Homing, for spawning, is indicated. This species tolerates wide ranges in temperature and salinities, however appears to be most common in waters where the salinity is between 20 and 36 ppt and where the temperature exceeds 25 C for at least one season of the year. Field observations and laboratory experiments indicate a preference for mud bottoms. The fish are nocturnal, burrowing during daylight, and the time of burrowing can be changed by artificial manipulation of day-length. Periods of submergence in excess of 24 hours occur and appear to be related to a combination of cold temperatures and short photoperiod or sudden cooling of the water temperature. Midshipmen are sensitive to low intensity light from at least far red to blue.

Bioluminescence appears to be used during courtship and as a warning device to predators. A poisonous opercular spine is not only painful to humans, but also can incapacitate other fish for

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[1] Modified from a dissertation presented to the graduate school, The University of Texas, in partial fulfillment of the requirement for the degree of Doctor of Philosophy, July 1966.

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up to half an hour. Spawning around Port Aransas reaches peaks during spring and summer in bays, and autumn in the Gulf. The fish spawn when one year old at a standard length of 100–120 mm; few midshipmen reach two years of age and almost all of these are males. Females average 140 eggs, which are up to 5 mm in diameter. Young are free swimming when 12–15 mm, and most of those spawned in bays move to the Gulf between late May and September.

Atlantic midshipmen feed largely on small crustacea (Amphipods, Mysidacea) and larval to juvenile *Anchoa*. Few parasites were found on the fish examined.

The length-weight relationship is described by the formula $W = 8.85\ (10^{-6}) \times L^{3.139}$ where W is weight in grams and L is standard length in millimeters. Examination of length frequencies and otoliths indicate that growth in length is essentially linear throughout most of life and may be expressed as $L = 21.938 + 7.76T$, where L is standard length in millimeters and T is time in months.

INTRODUCTION

The Atlantic midshipman, *Porichthys porosissimus* (Valenciennes), a common fish in the northern Gulf of Mexico was studied with respect to its systematics, morphology, behavior, bioluminescence and ecology.

The study area included Aransas and Copano Bays, Aransas Pass (the main migratory route for those fish in Aransas Bay) and the Gulf of Mexico from Galveston to Port Mansfield, Texas. Gulf material was from the shore line to a depth of 75 fathoms and concentrated off Aransas Pass between 0 and 25 fathoms (Figs. 1 & 2). Approximately 2500 specimens were collected from August 1964 through November 1965.

Three aspects of the biology of the species have previously been studied; the systematics, bioluminescence and kidney function. Hubbs and Schultz (1939), who reviewed the systematics of the genus *Porichthys*, included a comprehensive review of earlier systematic works. No subsequent work has been published on the systematics of *Porichthys porosissimus*; however, Gilbert (1967) *pers com* has a paper in press which reviews the systematics of the Atlantic and Gulf *Porichthys* and Fingerman (1959) wrote an unpublished M.S. thesis on the subject. Most bioluminescence work has been done on the Pacific forms, but Springer (1957) and Shoemaker (1957) have published notes on bioluminescence in *P. porosissimus*. Regional faunal works list the species but few contain any ecological data.

MATERIALS AND METHODS

Most specimens were captured in bottom trawls made in Aransas and Copano Bays and the Gulf of Mexico off Port Aransas on the M/V Lorene. The trawl used from August 1964 through October 1964 measured 6.7 m from the cork line to the cod end including the 1.2 m bag, and was 5.8 m wide at the mouth. It was 0.9 m deep from cork line to lead line while lowered, and was equipped with a "tickler chain" between the 0.6 × 1.5 m otter boards constructed of weighted plywood. The entire trawl, except the chafing gear, was constructed of ¾-inch stretch (⅜-inch bar) mesh nylon netting. A ¼-inch weave nylon mesh linear was used over the cod end to facilitate the capture of small fish. From November 1964 through November 1965 a trawl having similar dimensions to those of the above was used. The only differences were a more-heavily weighted lead line and more floats on the cork line. In the bay the trawl was usually dragged for 15 minutes; 10 minutes during the diel collections and when large catches of jellyfish filled the net. In the Gulf the trawl was dragged either 15 or 30 minutes. Trawling speed was approximately 1½ to 2 knots. The trawls were con-

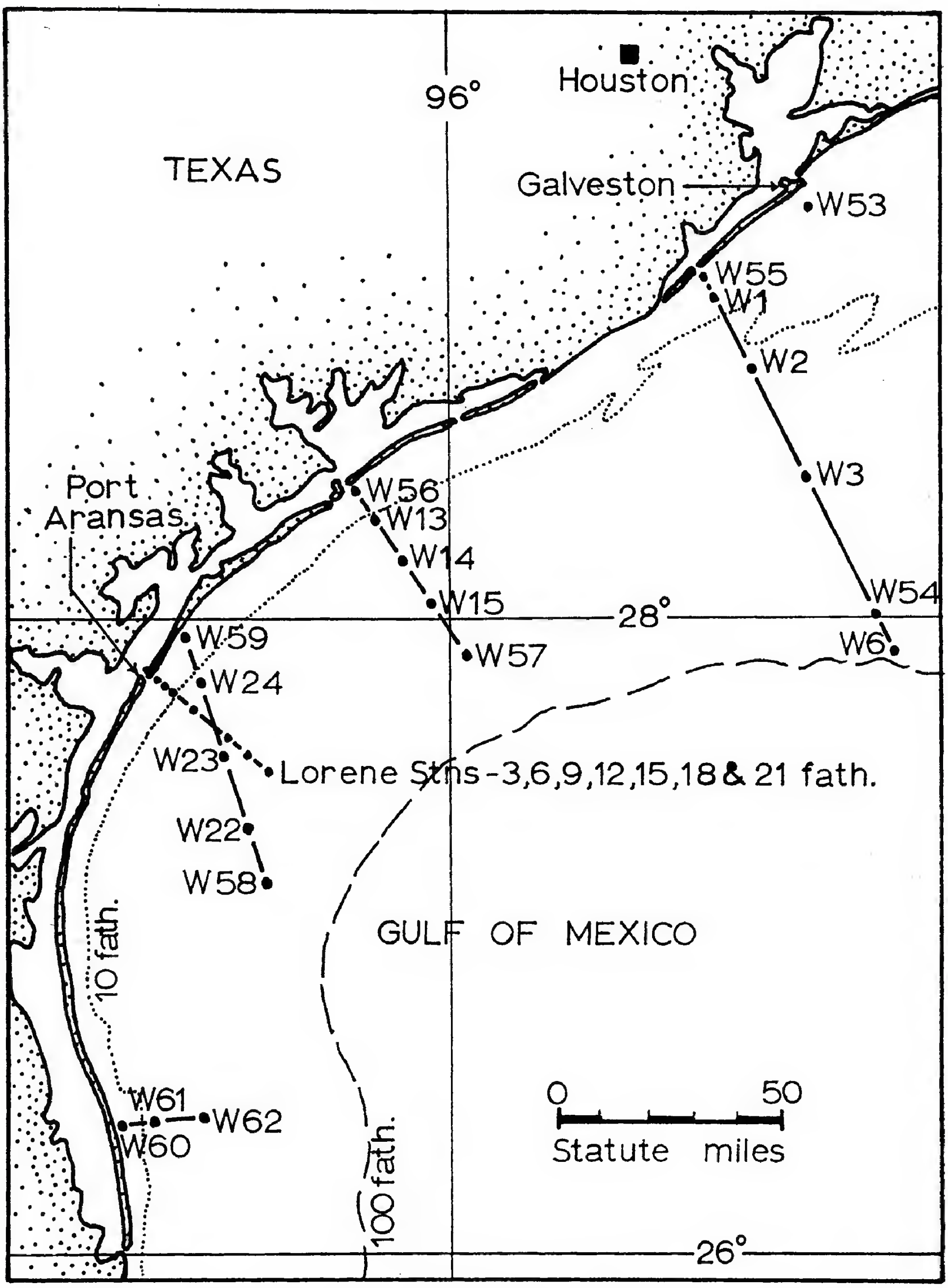


Fig. 1. Stations trawled in the Gulf of Mexico. Stations where the number is preceded by W were trawled from the M/V Gus III. Lorene stations were trawled from the M/V Lorene.

ducted during both day and night, twice a month in the bays and occasionally in the Gulf.

Other Gulf trawling was done from the M/V Gus III. Fishing stations varied in depth from 4 to 75 fathoms. The net was a 44-foot (13.4 m) standard shrimp

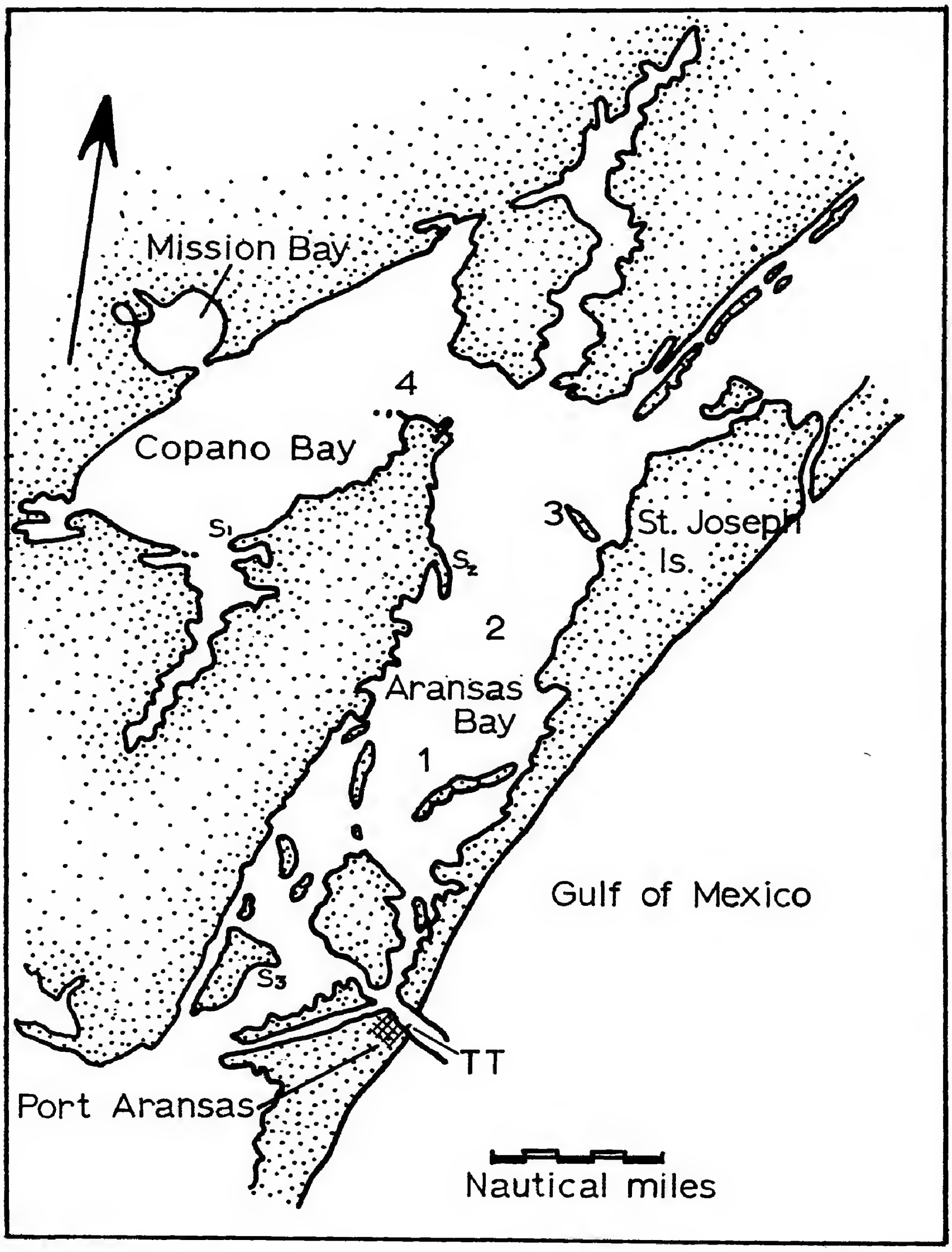


FIG. 2. Collecting stations in Aransas and Copano Bays. 1 to 4 were trawl stations; S_1 to S_3 were seine stations; TT is the site of the tide trap in Aransas Pass at the Institute of Marine Science.

otter trawl with a stretch mesh of 2 inches and was equipped with chafing gear. Trawling time was one hour at a speed of 2–3 knots. Stations were trawled at varying times of the night or day.

A tide trap, fixed under the University of Texas Marine Science Institute pier laboratory in Aransas Pass, was used to capture fish in the Pass on both ebb and flood tides. The trap, 1.59 m deep and 2.25 m wide, was made of ½-inch stretch nylon mesh for the first 6 m and ¼-inch nylon weave mesh for the remaining 2 m. The trap was raised and lowered by sliding the net on two parallel pipes secured vertically to the pier. Fish moving with the tidal currents enter the trap and are held there by the current.

A beach seine, 9.1 m long with ¼-inch weave mesh and a bag of ⅛-inch weave mesh, was used during the spring of 1965 in Redfish, Aransas and Copano Bays. Occasionally, seining was also done on the Gulf beach and along the jetties.

Specimens from South West Florida were collected by the M/V Gus III and by the Florida State Board of Conservation.

Specimens were preserved in 4% formaldehyde (10% formalin) in sea water solution for 24 to 48 hours, washed in fresh water and stored in 40% isopropyl alcohol. Live fish used in experiments were placed in a live tank on board the vessel and brought to the laboratory, where they were held without feeding. Following some experiments the fish were kept eight months without food and were alive at the time of release. Laboratory investigations included bottom preference, diel and seasonal rhythms, color change, light sensitivity, general behavior and predator-prey relations. Preserved specimens were used for length and weight measurements, meristic counts and internal examination of stomach content, parasites and gonads. All catch per hour data are in terms of numbers; all lengths are standard lengths; all counts are total counts; and, on paired structures, the left side was counted.

More detailed procedures employed in various aspects of the study are discussed in the appropriate sections of the text.

SYSTEMATICS

Porichthys porosissimus is a member of the only recognized family (Batrachoididae) in the Order Batrachoidiformes (Berg 1947; Greenwood *et al.* 1966), or Haplodoci (Regan 1912; 1926). The nearest related order may be the Lophiiformes (Berg 1947); the two groups were united into a common order, Pediculati (Regan 1912). The Batrachoids are probably an offshoot of a percoid or prepercoid ancestor (Gregory 1933). Greenwood *et al.* (1966) place the Batrachoids in a prepercoid superorder, Paracanthopterygii, along with the order Percopsiformes, Gobiesociformes, Lophiiformes and Gadiformes. Ribeiro's (1915) suggestion that the midshipman, due to the presence of light organs, be separated into the family Porichthyidae has not been accepted (Hubbs and Schultz 1939).

Porichthys porosissimus has undergone little nomenclatorial change since it was first described as *Batrachus porosissimus* by Valenciennes in Cuvier and Valenciennes (1837). Girard (1855) placed it in the genus *Porichthys,* and this designation has generally been used, although Jordan (1919) separated the Atlantic and Pacific forms of this genus, referring the Atlantic form to *Nauto-*

paedium. This designation has been followed in only two contributions to Batrachoid taxonomy (Hubbs and Schultz 1939; Caldwell and Caldwell 1963), both of which cast doubt on the validity of *Nautopaedium*. A few other works also list *Nautopaedium* without discussion of generic validity. The principal reported differences between the two genera are the direction of the premaxillary teeth in the adults and the position of the palatine teeth. Reportedly, pre-maxillary teeth are directed slightly backwards in *Porichthys* and forward in *Nautopaedium* and the palatine teeth are confined to the front of the palatine bone in *Nautopaedium* and spread along the bone in *Porichthys*. Since most authors follow the *Porichthys* designation, and since the tooth direction character is not consistent, the generic name *Porichthys* is used here.

The species name *porosissimus* appears to have been used in all work except Jordan and Gilbert (1882) who described a fish as *P. plectrodon* from Galveston, Texas. This species was synonymized with *P. porosissimus* by Jordan (1884). Both Fingerman (1959) and Gilbert (1967) *pers com* have recognized racial variations in *P. porosissimus*, the details of which are discussed in Morphological Variation.

DISTRIBUTION

The most northern reported specimen of *P. porosissimus* is from Cape Henry, Virginia, and the most southern specimen is from Necochea, Argentina (Hubbs and Schultz 1939). The deepest record is from 220 fathoms (M/V Oregon Stn. 4736; on February 29, 1964, *pers com* U.S.F.W.S.), but Gilbert (*pers com*) reports 175 fathoms as his deepest record. In this study juveniles were taken in two feet of water from Copano Bay, Texas (May 1965), this being the shallowest record. The M/V Oregon and Silver Bay obtained 90% of their recorded midshipman catch from less than 50 fathoms; hence, the depth preference appears to be more or less uniform over a large segment of the geographic range. A more detailed description of the distribution and movements within the study area is presented later.

MORPHOLOGICAL VARIATION

PIGMENT

Fingerman (1959) recognized three pigmentation patterns and Gilbert (*pers com*) notes the pigment pattern variation between populations. Differences in pattern were found between fish from southwest Florida and the Texas coast (Fig. 3).

Because there is considerable variation in pigmentation between the fish from southwest Florida and the Texas coast, it was decided to test midshipmen from Aransas Bay (Stn. 2) to determine the limits within which they could vary their pigmentation during a short time.

Three aquaria (51 × 27 × 31 cm) were set up using natural day length and identical light intensities. One aquarium had a substrate of fine white quartz gravel of approximately 1–4 mm in diameter, another had a substrate of black volcanic glass gravel of similar diameter and the third, used as the control, contained a mixture of grey sand and mud taken from the fish's habitat. Neither temperatures nor salinities were controlled, the former fluctuated with the room temperature, the latter between 25 and 40 ppt, similarly in all aquaria. During the first experiment three adults were maintained in each aquarium for three

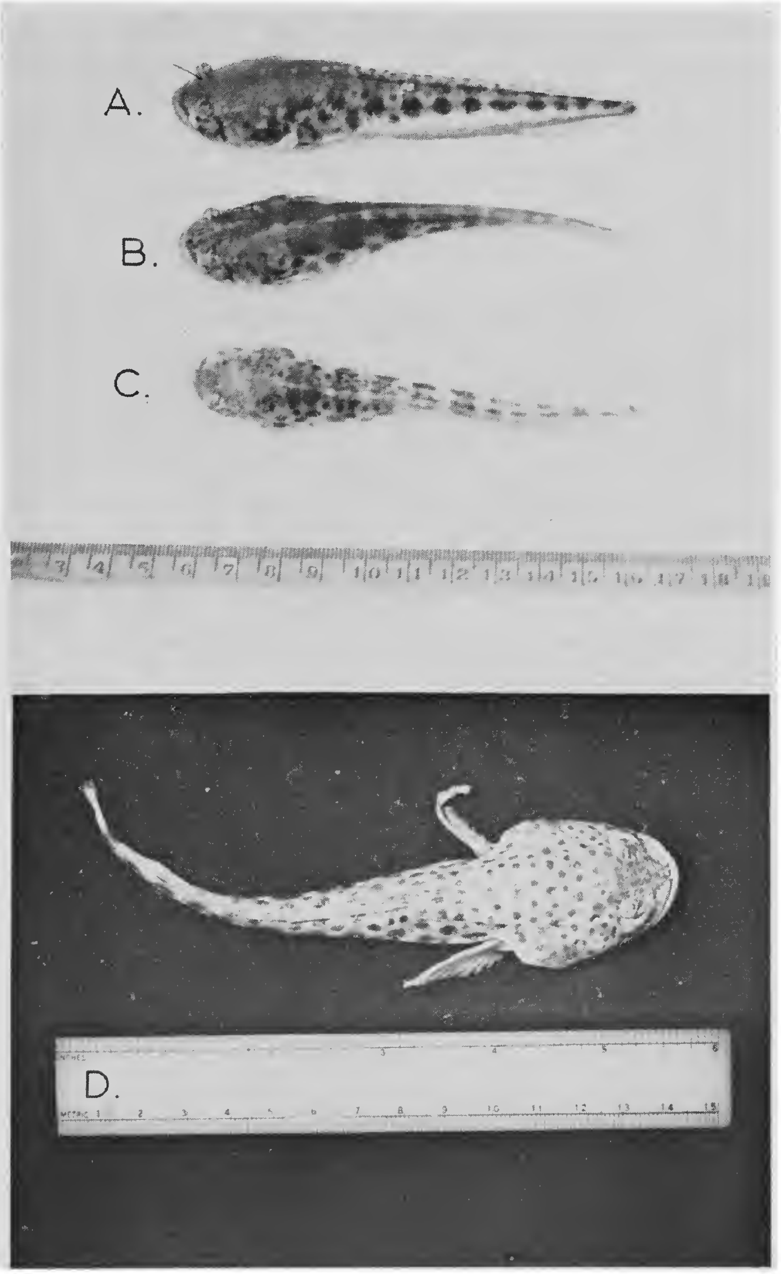


FIG. 3. Pigment variation in Texas and southwestern Florida midshipmen. A, B, and C—Texas fish; A—normal, B—from black substrate, C—from white substrate, D—normal southwest Florida fish.

weeks, during the second 32-day experiment five juveniles were held in the black and control tanks and twelve juveniles in the white substrate tank. Mortality during the experiments consisted of the death of one juvenile in the black tank after two weeks and of one juvenile which jumped out of the white tank.

In the first experiment the fish were not disturbed during the three weeks. Those in the white tank uniformly became lighter than those in either the control or the black tank (Fig. 3). In the second experiment it was found, by stirring fish from the substrate for observation, that differences in appearance among the fish in the tanks were becoming evident within two days of their introduction to the tanks.

At the end of the experiment (32 days) all fish were removed and the following color differences were noted: in the controls, coloration remained virtually unchanged throughout the experiment, *viz., dorsally* grey-brown, 9–11 grey brown bars running down the sides to about the mid-lateral position, and light golden yellow bellies; the black substrate fish were dorsally very dark grey-black with 9–11 dark bars as above but with grey inter-bar spaces darker than in the control, and the animals had deep golden yellow bellies; with the white substrate, ten fish were dorsally a lighter brown than both the control and black substrate fish, inter-bar spaces were very light grey and showed dorsally, there were white bellies in five fish, pale yellow bellies in five and one fish was scarcely different from the controls.

Although Aransas Bay fish became considerably lighter when subjected to a white substrate, they never reached the degree of lightness accompanied by complete spotting which is apparent in those from Key West, Florida (Fig. 3). Greatest contrast in the Texas fish occurred in the dark individuals.

In summary, fish from Aransas Bay, when placed on a black substrate, became slightly darker than the control fish; those on a white background for a short time (32 days) became lighter, but did not form the light and spotted appearance of the fish from the Gulf off Southwestern Florida. The difference in appearance may be genetic or an example of the phenomena of loss or gain of melanophores described by Marshall (1965).

MERISTICS

Hubbs and Schultz (1939) did not report geographical variation in the meristic counts from Atlantic midshipmen. Fingerman (1959) found statistically nonsignificant variation in meristic counts. By comparing fish of known age and spawning stocks I found significant differences in meristic counts contrasting fish from the Gulf with those from the bays around Aransas Pass, and both groups differed from the southwestern Florida fish.

Fish were collected for comparison from the following seven groups collected during 1964–65:

1) Southwest Florida, adults, winter 1964–65;
2) Gulf off Aransas Pass, young, hatched fall 1964;
3) Parents of 2 (Gulf adults);
4) Aransas and Copano Bays, young, hatched summer 1964;
5) Aransas and Copano Bays, spawning adults, spring 1965;

6) Aransas and Copano Bays, young, hatched spring 1964; and
7) Offspring of 5 (Bay young).

Counts were made of the numbers of dorsal, anal and pectoral fin rays and of the lateral row of photophores (Figs. 4, 5 and Table 1). The latter were not used in the analysis because it was found that lateral photophore number increased with size in the young.

It is noteworthy that in all fin ray counts analyzed the relative order is the same; southwestern Florida fish had the fewest rays, Gulf off Port Aransas next

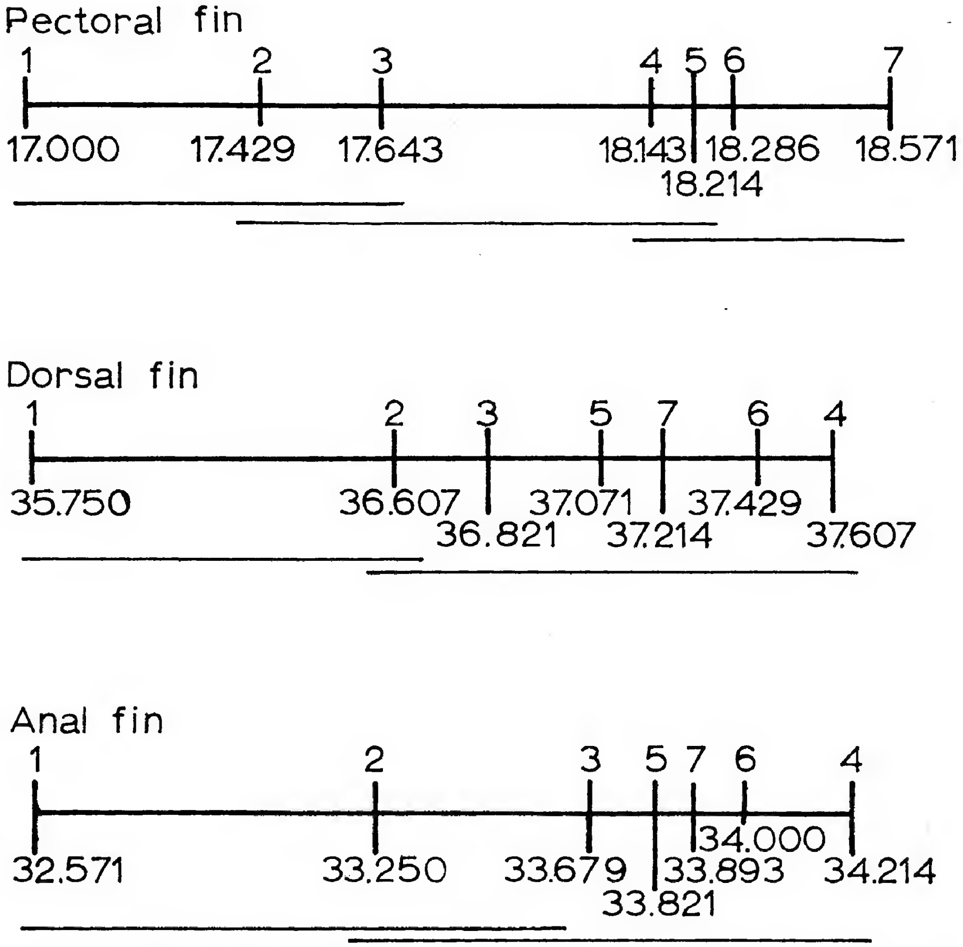


FIG. 4. Results of Duncan's F test on the fin ray counts in midshipmen from different areas. Numbers are means of 28 counts. Means not underscored by the same line are significantly different at the 95 % level.

1. Southwest Florida, adults, winter 1964–1965.
2. Gulf off Aransas Pass, young, hatched fall 1964.
3. Parents of 2. (Gulf adults).
4. Aransas and Copano Bays, young, hatched summer 1964.
5. Aransas and Copano Bays, spawning adults, spring 1965.
6. Aransas and Copano Bays, young, hatched spring 1964.
7. Offspring of 5. (Bay young hatched spring 1965).

and Aransas and Copano Bay fish had the greatest number of rays. The differences were tested for significance by Duncan's (1955) New Multiple Range and Multiple F test (Fig. 4) and were significant (5% level) using Freund's (1953) z-test (Fig. 5). The slight differences between Gulf parents and offspring and between bay samples can be attributed to sampling error and slightly different

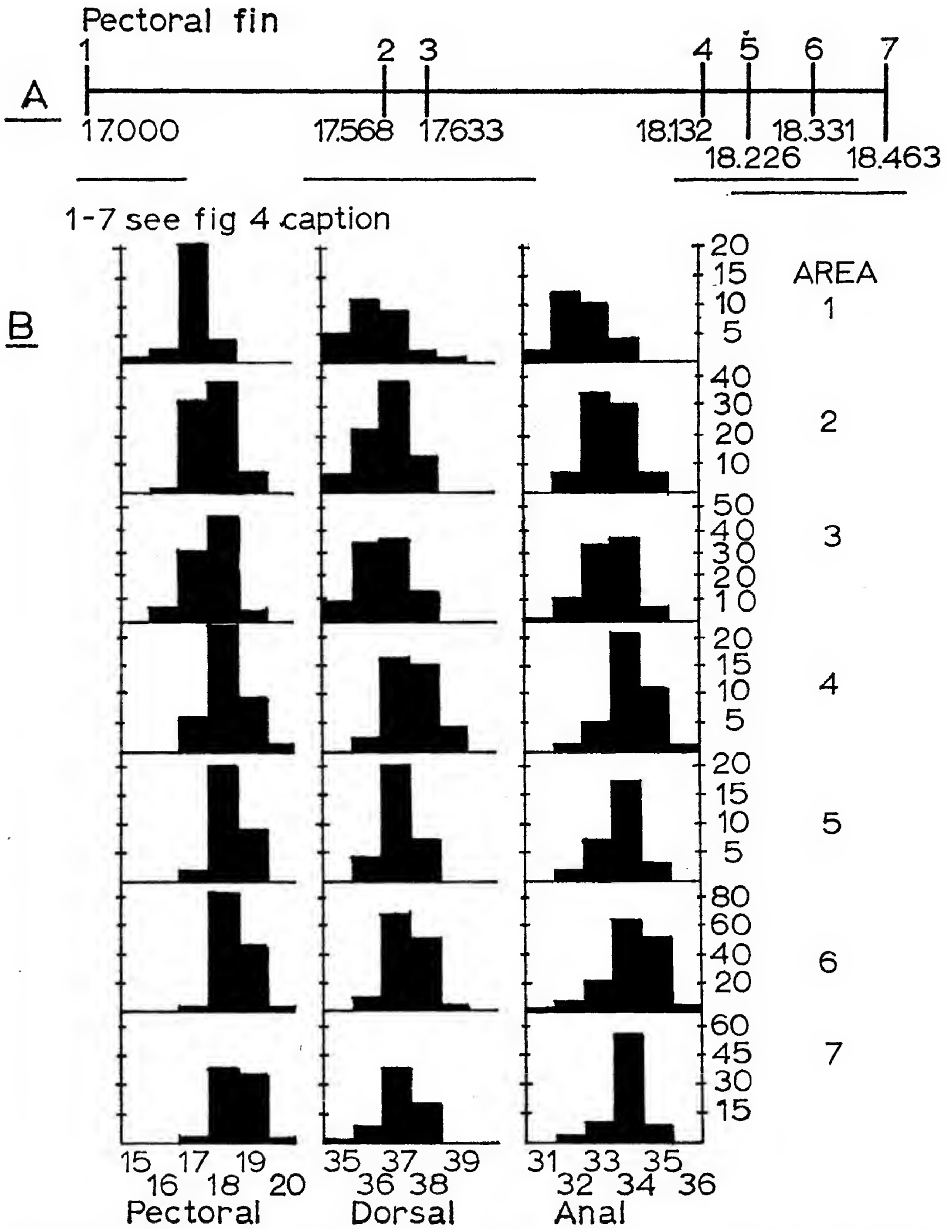


FIG. 5. A. Z-test on the pectoral fin ray counts. Numbers are means; total n = 484, individual group's n's varied (Table 1). B. Histograms are the distribution of all counts. The numbers 1 to 7 are as in Fig. 4.

hatching conditions. The significant difference between maximum and minimum bay counts may reflect the different environmental conditions in the summer and the spring.

The similarity within all Gulf counts, and within all bay counts, suggests homing to spawning areas.

The differences between the meristics and pigmentation of fish obtained from southwest Florida and from Port Aransas suggest the possibility of genetic difeferences between the two populations although probably not at the specific level. There are approximately 1200 statute miles along the coast separating the two areas, although populations of midshipmen exist between these areas. The differences between fish of the Gulf and bays in the Port Aransas area may be environmental (Täning 1944; 1950; 1952; Heuts 1947a; b; 1949; Lindsey 1954; 1958; 1962). In the Gulf off Port Aransas midshipmen spawn in the fall during dropping temperatures, but those in bays spawn in spring during rapidly increasing temperatures and in the summer during high fluctuating temperatures. Moreover, the salinity in the Gulf is more consistent than in the bays. Variation

TABLE 1

Meristic counts (fin rays)—ranges and means. Area numbers are as in text.

Area	Pectoral	Dorsal	Anal	
1 (Florida)	17.000	35.750	32.571	x̄
	15–18	34–37	31–34	range
	28	28	28	n
All Gulf	17.599	36.663	33.455	x̄
2 & 3	16–19	34–38	31–35	range
combined	167	166	167	n
All Bay	18.331	37.326	33.993	x̄
4,5,6 & 7	17–20	35–39	30–37	range
combined	287	271	285	n
2 (Gulf)	17.633	36.727	33.494	x̄
	16–19	34–38	32–35	range
	79	77	79	n
3 (Gulf)	17.568	36.607	33.420	x̄
	16–19	35–38	31–35	range
	88	89	88	n
4 (Bays)	18.132	37.553	34.156	x̄
	17–20	36–39	32–36	range
	38	38	38	n
5 (Bays)	18.226	37.097	33.839	x̄
	17–19	36–38	32–36	range
	31	31	31	n
6 (Bays)	18.331	37.387	34.015	x̄
	17–20	35–39	30–37	range
	136	135	136	n
7 (Bays)	18.463	37.179	33.938	x̄
	17–20	35–38	32–35	range
	82	67	80	n

in both salinity and temperature during early development is known to affect meristic counts in fishes.

MORPHOLOGY

The external anatomy of Atlantic midshipmen has been described by Jordan and Everman (1896), Hubbs and Schultz (1939), Fingerman (1959) and others (see frontispiece), therefore only the opercular spine and teeth will be discussed here. The opercular spine which points posteriorly and is curved dorsally appears to have a protective function. Normally, it is sheathed by skin except at its sharp point. The fish can erect the spine so that it is at an approximate 45 degree angle dorso-laterally from the midline. When in this position the skin sheath has slid back about halfway, and can be easily moved further back exposing three-quarters of the spine. The spine is pointed ventrally and grooved dorsally, with flat lateral sides. The groove widens at the dorsal base of the spine and contains a yellow pulpy mass, which may be the poison gland. The poison probably travels from the gland up the groove into the victim. (Effects of the toxin are discussed later). Greenwood *et al.* (1966) illustrated the skull musculature of *P. porosissimus*. Their figure shows the muscles responsible for erecting the spine.

Hubbs and Schultz (1939) described the sexual dimorphism in the teeth of adults. Generally, adult males' posterior premaxillary teeth are large and directed forward; adult females' are smaller and not directed forward. The characteristic, however, is valid only in adult reproductive males, and there is some overlap in the tooth size of the sexes. Approximately 5% of adult females I examined had fairly large forward directed teeth while 10% of adult males had smaller straight teeth.

In describing the internal anatomy of Atlantic midshipman, Fingerman (1959) found the peritoneum to be black in all specimens, while Hubbs and Schultz (1939) found it to be white in adults. I found the peritoneum to be black in all sizes.

In the specimens I examined, the walls of the swim bladder were quite thick, up to 1.2 mm, especially in the dorso-lateral and ventro-lateral regions. Greene (1924) described in some detail the structure and function of the swim bladder in a Pacific form, *P. notatus*. The structure of the bladder in *P. porosissimus* appeared to be essentially the same. Judging from Greene's Figures 1, 2 and 3, they appear to differ in *P. porosissimus* in that the anterior ends are more pointed, the lateral muscle layer is thinner and the lateral walls are thicker. Greene believed that the swim bladder of *P. notatus* had hydrostatic and vocal functions; it is likely, on the grounds of morphological similarity, that the swim bladder in *P. porosissimus* has similar functions.

The gonads lie posterio-ventrally in the gut cavity extending to fill approximately one-half to one-third of the space in ripe females and one-third to one-quarter of the space in ripe males.

The midshipman has an aglomerular kidney. Friedman and Kaplan (1941) and DiFiore (1948) have described its structure and function in both Pacific and Atlantic *Porichthys*.

Greene (1899) first described the micro-anatomy of the light organs in *P.*

notatus. Nicol (1957) decribed these organs in *P. myriaster* and was the first to show neural connections to the photophores. Although these two species are both Pacific forms, it is probable that the Atlantic midshipman is very similar.

Five specimens were used for osteological examination. They were cleared and stained with alizarin (Holister 1934). The anatomy of the skull of *Porichthys* was briefly discussed by Berg (1947) and Regan (1912), and the ethmoid and shoulder girdles were discussed by Stark (1926; 1930). Gregory (1933) illustrated and described in detail the skull of another batrachoid, *Opsanus tau* (Linnaeus). Skulls of *P. porosissimus* differ from these previous descriptions in some respects.

The dorsal and lateral views of the skull of *P. porosissimus* are almost identical to those shown in Figures 263A and B of Gregory (1933) for *Opsanus tau*. In Atlantic midshipmen the vomer does not protrude beyond the premaxillary bone, and in dorsal view the dentary is "U" shaped. In *P. porosissimus* the hyomandibular is narrower and extends further ventrally; the articular bone does not extend so far posteriorly, and there are differences in shape of the quadrate-pterygoid series. The Atlantic midshipman has a single opercular spine (Greenwood *et al.* 1966). The teeth are sharp rather than short and conical, and the vomer is notched in the genus *Porichthys*. In ventral view the mandible and lower hyoid arch form a "V" in *Opsanus* and a "U" in *Porichthys*. The branchial apparatus is rather large in *Porichthys*.

Previous authors have commented on the similarity in the caudal skeleton of the Batrachoids and Salmopercae (Percopsiformes), and Regan (1912) suggested that it may indicate the evolution of Batrachoids from a prepercoid stock. Gregory (1933) suggested that the similarity may mean divergence from the "acanthopt" stock before the beginning of the modern percomorph families. It can be seen in the drawing of the caudal skeleton of *P. porosissimus* in Figure 6 that the dorsal hypural (HPI) is a development of the last vertebra. Greenwood *et al.* (1966) have noted the same structural arrangement, but interpret it to result from the upper hypural being ankylosed to the last half centrum. The differences in interpretation do not distort the possible phylogenetic conclusion of a prepercoid ancestry for the batrachoid fishes.

HABITAT DESCRIPTION

MATERIALS AND METHODS

The methods of collection are previously described. During work in the bays and in the Gulf of Mexico from the M/V Lorene, water temperatures were measured at the surface and bottom, bottom water samples were taken in a Frausche bottle. Salinity measurements were made in the laboratory at room temperature with an American Optical, Temperature-Compensated Refractometer (*see* Behrens 1965). Dissolved oxygen was measured using the modified Winkler technique. Surface and bottom light was measured with a submarine photometer. Bottom mud samples were analyzed for particle size by settling as described in Folk (1964).

On the M/V Gus III, temperature and salinity data were taken by the U. S. Bureau of Commercial Fisheries, Galveston Laboratory. Bottom temperatures were taken one meter above the bottom by bathythermograph. Salinities were titrated from water samples also taken one meter above the bottom. More details of these data can be obtained from Gulf Shrimp Program, U.S. Bureau of Commercial Fisheries, Galveston, Texas. Because the Atlantic midshipman is a bottom dwelling fish, bottom conditions are emphasized in subsequent discussion.

BAYS

Texas coastal bays are large areas of shallow saline waters separated from the Gulf of Mexico by a chain of barrier islands (Fig. 1). Passes between the islands give some of the bays direct water exchange with the Gulf of Mexico. Sampling stations were set up in Aransas and Copano Bays (Fig. 2). Aransas Bay is a primary bay, *viz,* emptying directly into the Gulf of Mexico; and Copano Bay is a secondary one, emptying into Aransas Bay.

No major river system empties into Aransas Bay. The Aransas River enters the western tip of Copano Bay, and the Mission River enters Copano Bay via Mission Bay. According to Copeland (1966) 1.4 acre-feet surface-acre^{-1} year^{-1} of fresh water enters Aransas and Copano Bays. Gulf water entering Aransas Bay via Aransas Pass and Lydia Ann Channel is sufficient to maintain polyhalinity, which varies with freshwater input. Laguna Madre to the south is an area of hypersalinity and San Antonio Bay to the north is an area of hyposalinity.

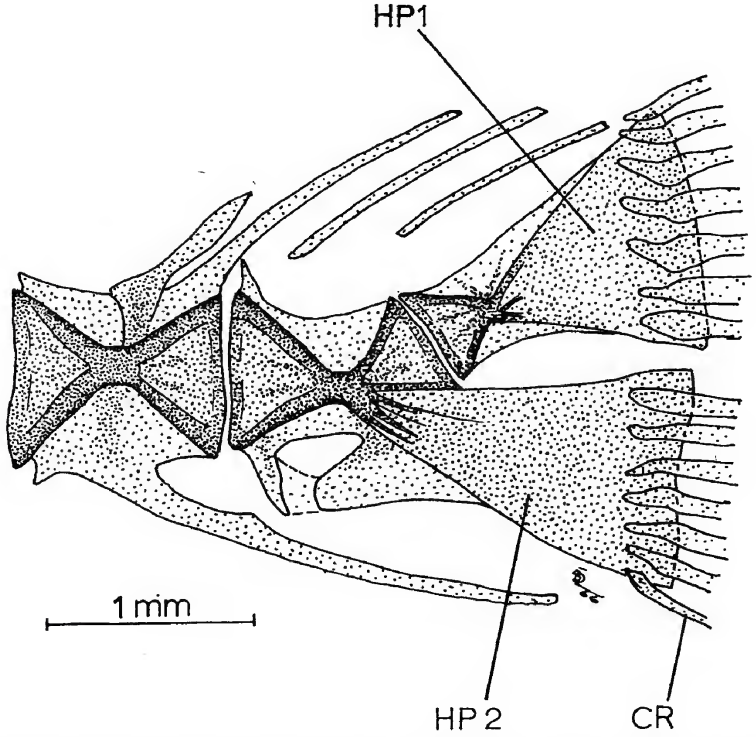


FIG. 6. Caudal skeleton of *P. porosissimus.* HP1 = dorsal hypural (note it is formed from the ultimate vertebra). HP2 = ventral hypural (note its origin on the penultimate vertebra). CR = caudal ray.

Aransas and Copano Bays are about the same size and total about 127,000 acres (Copeland 1966). The maximum depth of Aransas Bay is approximately four m, though most of the Bay varies between 2.5–3.5 m. The maximum depth of Copano Bay is approximately three m, with most of the Bay being 1.5–2.5 m. Both bays have extensive shallows of one m or less around their perimeters and many shoals and reefs of less than one m depth. The bottom of Copano Bay is composed of areas of grey mud and areas of oyster reefs. Aransas Bay has a similar bottom composition except that it also has areas of fine sand. A more detailed description of these bays may be found in Gunter (1945).

The positions and depths of the four stations in Aransas and Copano Bays were:

Station 1—N.E. of marker 69 on the intracoastal canal, depth 2–2.6 m;

Station 2—at marker 49 S.E. of canal, depth 3.0–3.6 m;

Station 3—at marker 31 S.E. of canal, depth 2.5–3.3 m; and

Station 4—in Copano Bay immediately S.E. of Lap Reef, depth 1.8–2.5 m.

During the summer the temperature and salinity at the bay stations were both high, during the winter they were both low (Fig. 7). In the spring the temperature rose before the salinity, a probable result of considerable rainfall in the spring of 1965. The sudden salinity increase in March may be related to the spring high tides. The effect did not extend as far as Copano Bay. The most severe fluctuations in temperature and salinity, which occur in the fall (October, November, December), coincide with cold north winds, which are usually accompanied by a rain and followed by warm dry periods. Biological effects (fish kills) due to severe cold north winds have been discussed by Gunter (1941).

The summer increase in salinity in the bay is associated with high tides in August and September and invasion of saline Gulf water. The increase was least felt in Copano Bay, the farthest from the Gulf. However, the position and depth of Copano Bay would make it more sensitive to loss of water through evaporation. Interaction between rainfall, evaporation and influx of Gulf water tends to produce complex salinity relationships. Copano Bay is most likely to become fresh and Station 1 least likely.

There was little variation in temperature and salinity over a 24-hour period on those days that both were measured (Fig. 8). The variation that occurred was probably caused by water mass movement (tidal) and normal daily temperature fluctuations. Small differences occurred between the surface and the bottom with respect to temperature and salinity indicative of complete mixing of the water by wind action. Minor temperature (less than 0.2 C) difference between the surface and bottom could be attributed to evaporative cooling or radiant heating.

The oxygen content of the water in the study area of Aransas Bay is almost always near air saturation (Fig. 9) and there usually is sufficient wind to prevent stratification. Most oxygen is added by wind-caused agitation. This is in contrast to the shallow, heavily vegetated areas in the bays (Odum 1963).

Hourly determinations of bottom and surface light intensities during the diel periods (Fig. 9) indicate that 1/10th to 1/150th of the incident surface light reaches the bottom during the day (1030 hours to 1730 hours) dependent upon turbidity. Conditions found in October and April can be termed average (wind

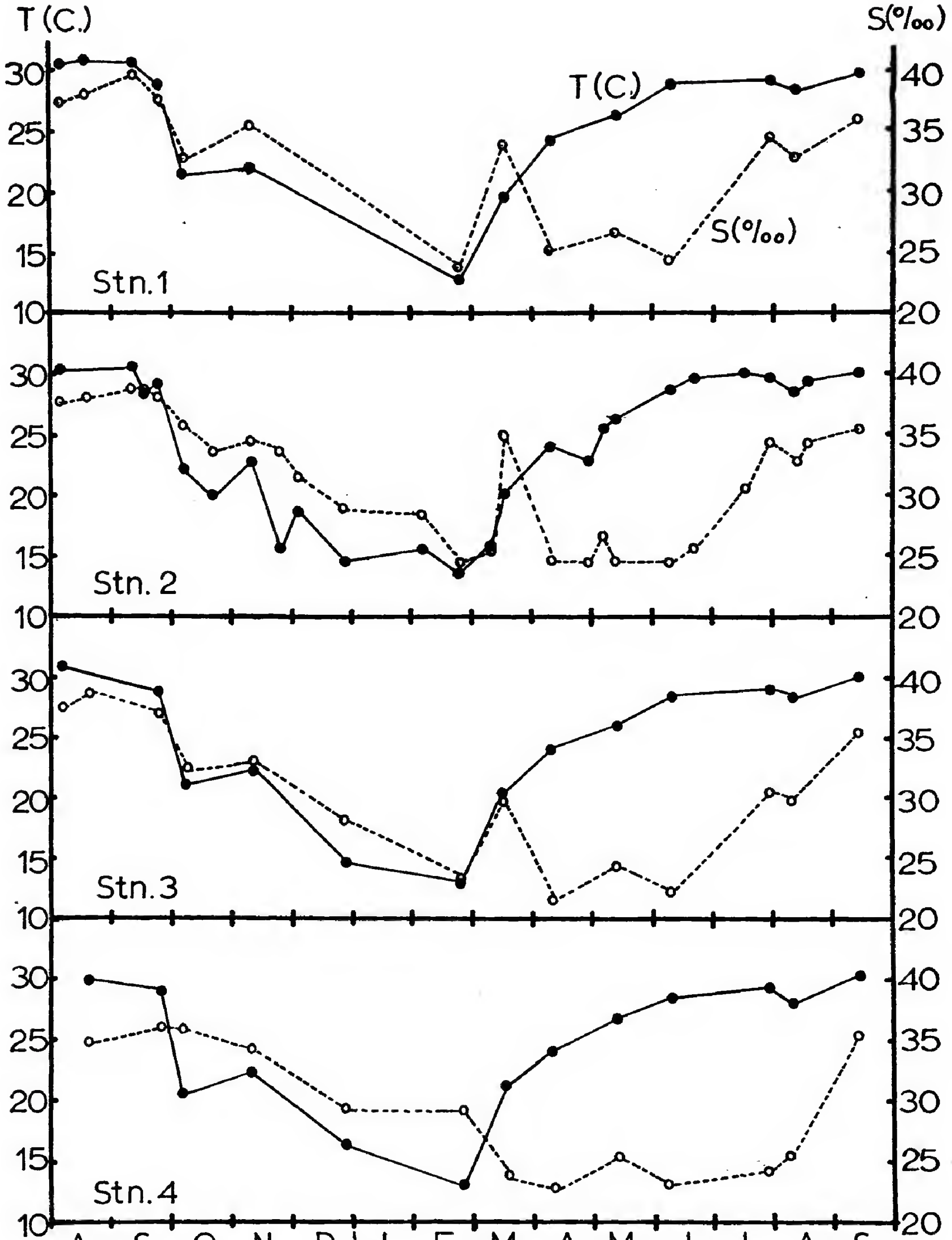


Fig. 7. Bottom temperature and salinity profile at the trawl stations in Aransas and Copano Bays from August 1964 to September 1965. When more than one temperature or salinity was taken on any one day the average is shown.

velocity approximately 15 m.p.h., water choppy, water moderately turbid) and during this time approximately 1/60th of the surface light was recorded at the bottom. The night of February 7 was calm and during the next day 1/10th of the surface light was recorded at the bottom. During the night of August 7–8 the wind velocity was high, 20–25 m.p.h.) and a large choppy sea was running; the next day only 1/150th of the surface light was recorded at the bottom.

Bottom sediment analysis is shown in Figure 10. Stations 2, 3 and 4 were similar, the bottom being mainly clays and silts (particle size <0.0156 mm) while station 1 was largely shell particles with a particle size greater than 0.063 mm. A

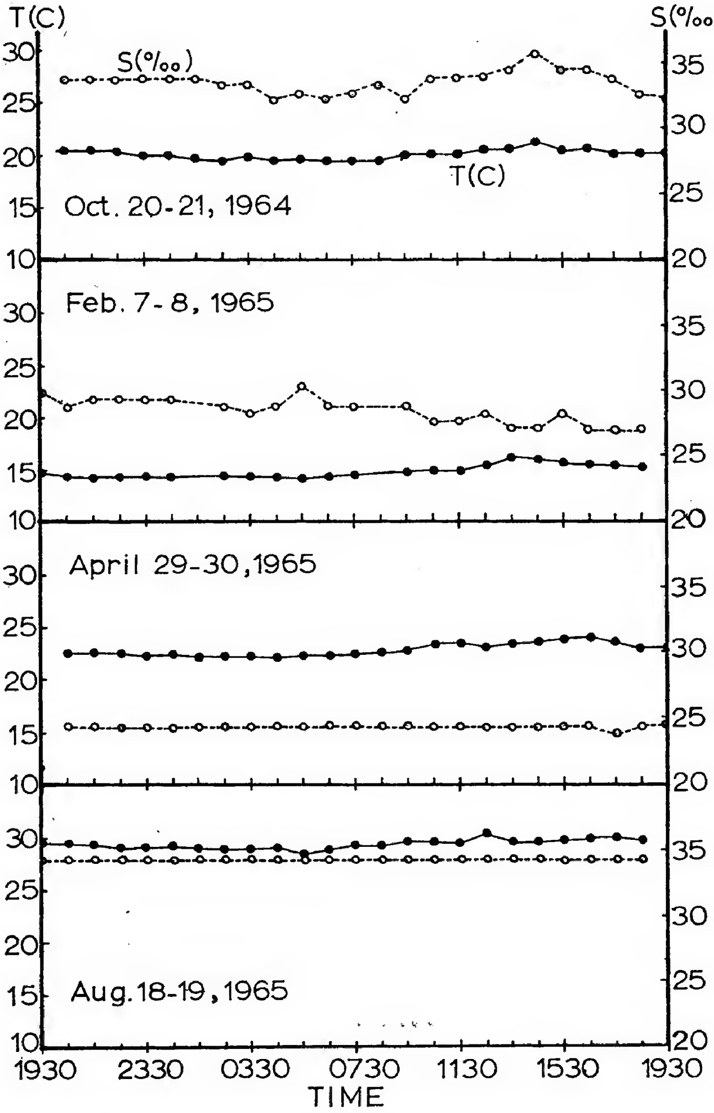


FIG. 8. Diel variation in bottom salinity and temperature at station 2 in Aransas Bay.

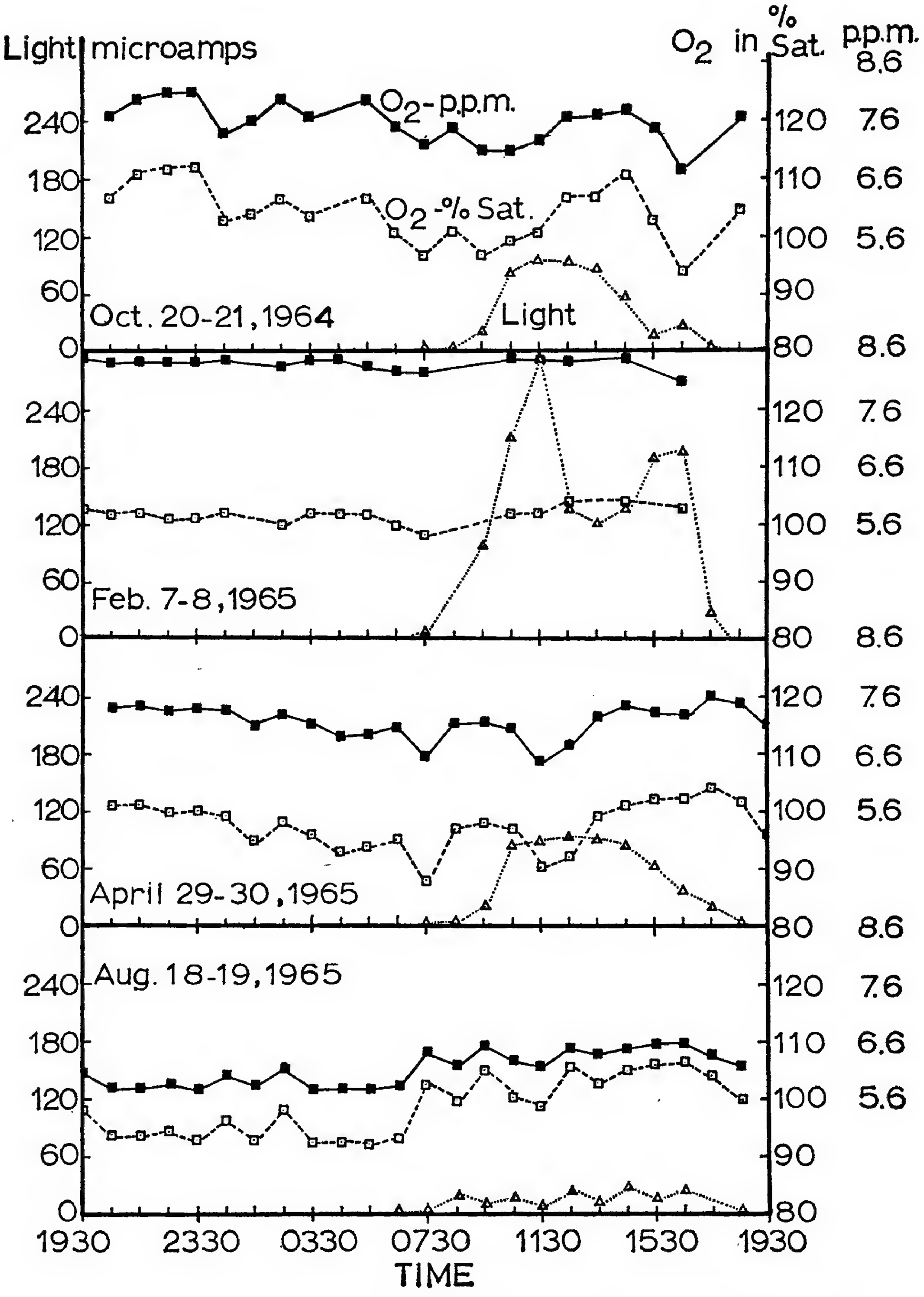


FIG. 9. Diel variation in bottom dissolved O_2 and light at Aransas Bay station 2.

moderate number of siliceous worm tubes were encountered in the mud from station 3; no worms were seen in the tubes.

Mud temperatures were recorded at station 2 (Fig. 11). During the cold winter the mud was 1 C higher temperature at approximately 30 cm below the mud-water interface. Similar but reverse conditions (mud cooler) exist during the summer. The mud appears to be a somewhat more constant thermal environment than the water above it.

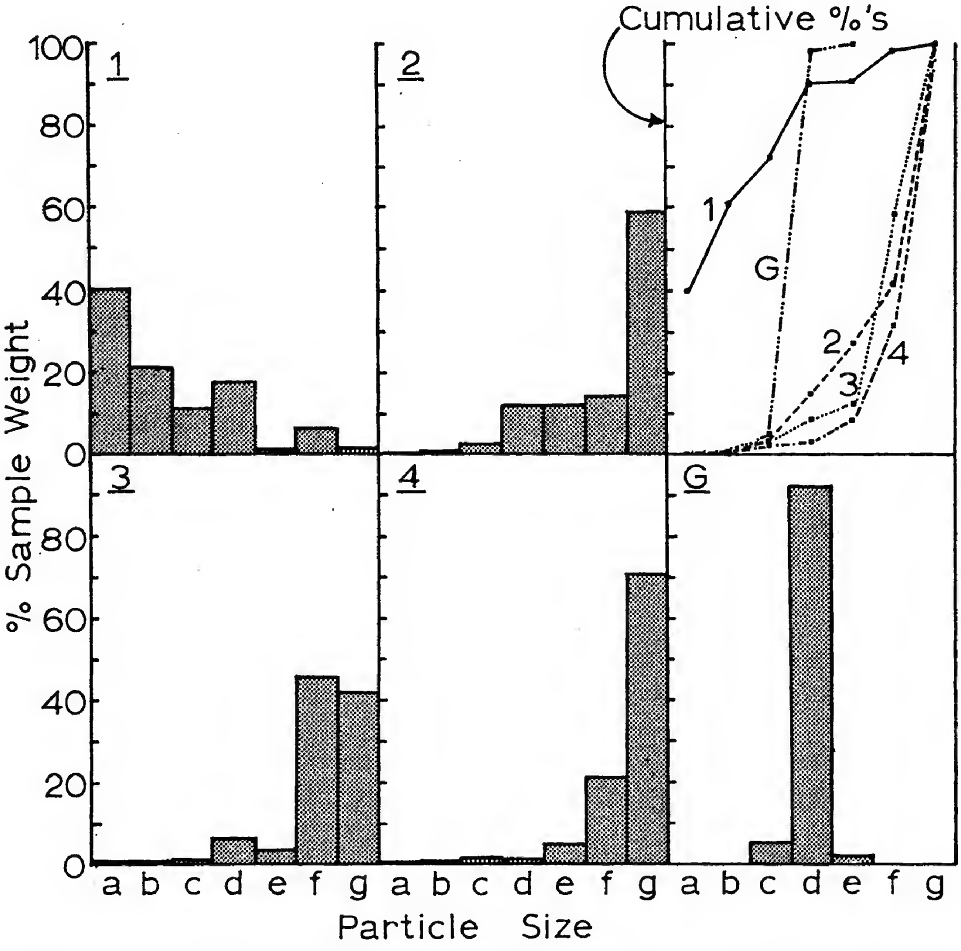


FIG. 10. Particle size analysis from the bottom sediments at stations 1, 2, and 3 in Aransas Bay, station 4 in Copano Bay and the Gulf Beach (G.).

Particle size key
a = larger than 2.00 mm
b = 0.50 to 2.00 mm
c = 0.125 to 0.50 mm
d = 0.063 to 0.125 mm
e = 0.0156 to 0.063 mm
f = 0.0036 to 0.0156 mm
g = smaller than 0.0036 mm

GULF

The Gulf of Mexico off the Texas coast slopes very gradually to the edge of the shelf. Off Port Aransas the slope is roughly one mile offshore for each fathom of depth. Gulf collection stations are shown in Figure 1. Generally speaking, the near shore area of the Texas coast (out to 6 fathoms, 11 m) has a sandy bottom (Fig. 10). Farther off-shore alternating sand and mud are encountered and finally only mud. Curry (1960) mapped and discussed the sediments in the shallow Gulf.

The bottom types at the individual stations tended to have more fine particles at a farther distance offshore (Table 2). The greatest fluctuation in bottom temperature and salinity occurred at the shallower Gulf stations (Figs. 12 and 13). The fall-winter drop in temperatures was most rapid and reached a minimum earlier at the shallow stations. Similarly, the spring warming was most apparent in the shallow water. Salinity was virtually constant at the deepest station in each transect; however, fluctuations in temperature are apparent at 60 fathoms (109.7 m).

Jones *et al.* (1965) discussed in detail the hydrography of the shallow Gulf off Aransas Pass and gave data on the light penetration and water mass distinctions in the area.

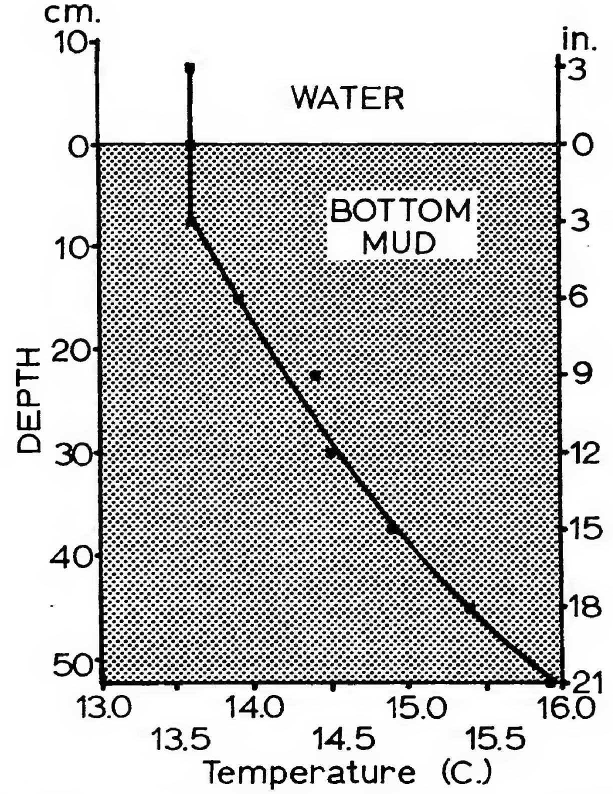


Fig. 11. Mud temperatures at Aransas Bay station 2—winter. (Feb. 25, 1965).

SUMMARY

Bay habitats in the study area had a bottom of fine silt and clay sediment, except station 1, which is on a dead oyster reef. The bottom water temperature during the study period varied between 32 and 12 C. Salinity varied between 39 and 20 ppt, roughly following the temperature pattern. Sudden drops in water temperature occurred during very cold winter winds. There was a short time lag between rainfall and drop in bay salinity. Dissolved oxygen was always close to saturation. The bays are generally turbid, approximately 1/60th of the surface light reaching the bottom at 3 m on most days.

In the Gulf the bottom sediments vary with depth, generally sand near shore and mud farther out. Bottom temperature varies with depth; inshore it varied between 30–13 C, at 15 fathoms (27.4 m) between 30–15 C, at 25 fathoms (45.7 m) between 28–17 C and at 60 fathoms (109.7 m) between 20–15 C. Salinity varied most inshore and was between 24 and 37 ppt; little variation was found farther offshore where salinity was approximately 36.5 ppt. Turbidity varied with season and was greatest inshore.

Table 2

Depth and bottom type—Gulf stations

Station	Approximately Lat.	Long.	Depth		Bottom type (from Curry 1960)
Off A.P.[1]	27° 49′	97° 00′	3 fath.	5.5 M	Sand
W 53	29° 16′	94° 47′	4 fath.	7.3 M	Sand
W 55	29° 03′	95° 07′	4 fath.	7.3 M	Sand
W 56	28° 24′	96° 21′	4 fath.	7.3 M	Sand
W 59	27° 57′	96° 54′	4 fath.	7.3 M	Sand
W 60	26° 24′	97° 10′	5 fath.	9.1 M	Sand
Off A.P.	27° 47′	96° 58′	6 fath.	11.0 M	Sand and silt
W 1	29° 00′	95° 05′	7.5 fath.	13.7 M	Sand, silt and clay
W 13	28° 19′	96° 17′	7.5 fath.	13.7 M	Clay and silt
W 24	27° 47′	96° 52′	7.5 fath.	13.7 M	Sand and silt
Off A.P.	27° 45′	96° 56′	9 fath.	16.5 M	Sand and silt
Off A.P.	27° 42′	96° 53′	12 fath.	21.9 M	Clay and silt
W 61	26° 25′	97° 02′	12 fath.	21.9 M	Sand and clay
W 23	27° 34′	96° 47′	15 fath.	27.4 M	Clay and silt
W 14	28° 12′	96° 12′	15 fath.	27.4 M	Sand, clay and silt
W 2	28° 45′	94° 58′	15 fath.	27.4 M	Sand, clay and silt
Off A.P.	27° 37′	96° 47′	15 fath.	27.4 M	Clay and silt
Off A.P.	27° 34′	96° 42′	18 fath.	32.9 M	Clay and silt
Off A.P.	27° 32′	96° 39′	21 fath.	38.4 M	Clay and silt
W 62	26° 26′	96° 52′	25 fath.	45.7 M	Clay and silt
W 22	27° 20′	96° 42′	25 fath.	45.7 M	Clay and silt
W 15	28° 03′	96° 05′	25 fath.	45.7 M	Sand, clay and silt
W 54	28° 00′	94° 32′	40 fath.	73.2 M	Sand, clay and silt
W 6	27° 53′	94° 28′	60 fath.	109.7 M	Sand, clay and silt

[1] A.P. is Aransas Pass.

HABITAT PREFERENCE

The midshipman tolerates wide ranges of salinity. It has been found in salinities ranging from 1.2 ppt (Renfro 1959) to 44.5 ppt. (Hoese 1960). Its occurrence at intermediate values of salinity has been recorded by Gunter (1945), Pullen (1960) and Stevens (1960). I never found the animal outside the above ranges of salinities, and it seemed to be most abundant in water between 20 and 37 ppt. It has never been reported in fresh water or in the hypersaline Laguna Madre. In an experiment in which midshipmen were held in tanks, when the

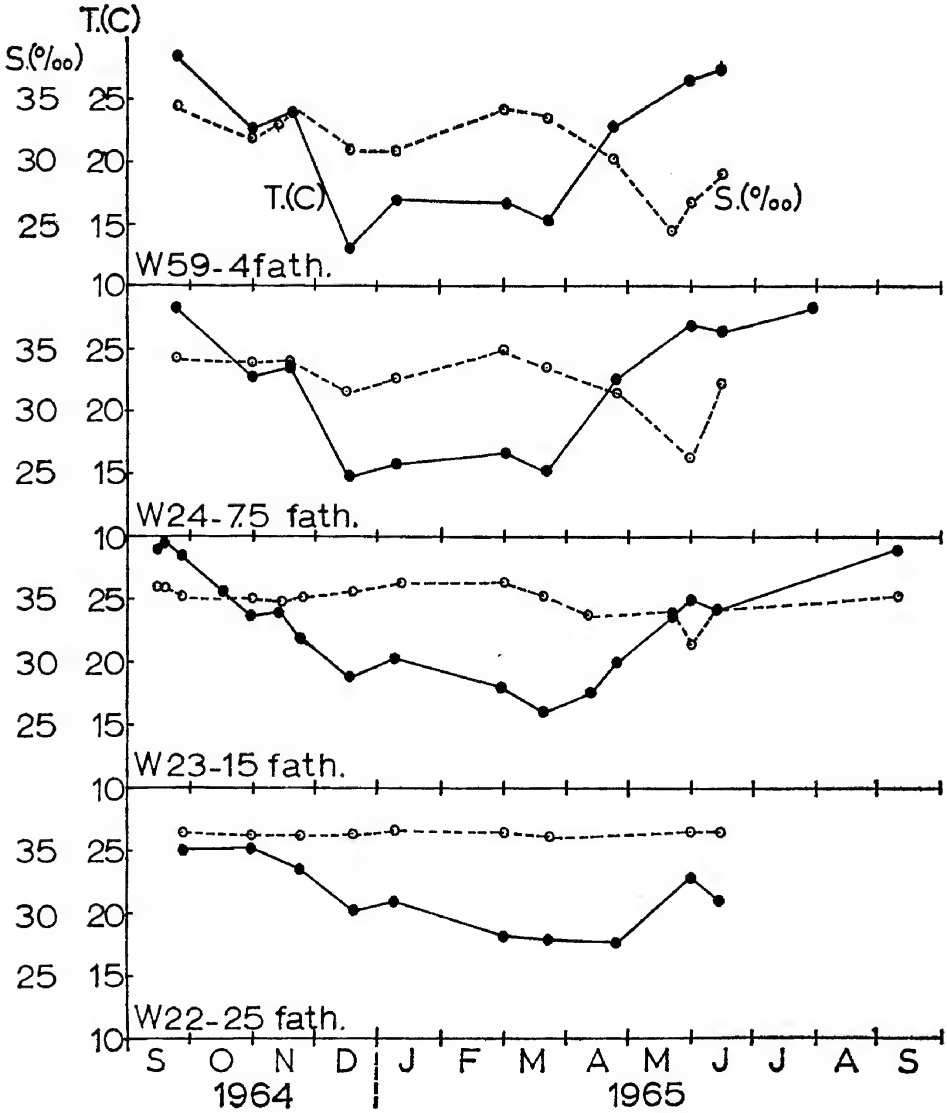


Fig. 12. Bottom temperatures and salinities at different depths in the Gulf of Mexico off Aransas Pass Sept. 1964 to Sept. 1965.

salinity was lowered suddenly from about 35 to 24 ppt no effect on behavior or survival was noticed.

Midshipmen are also tolerant of a wide range of temperature. Their range encompasses both tropical and warm temperate waters. In this study they were found in waters of 12.9 to 31.7 C; the maximum temperature is higher than those previously reported. Pullen (1960) recorded them in 11 C water in Galveston Bay. In the laboratory, Atlantic midshipmen were exposed to 8.5 C during two successive nights and no mortality ensued. Also, it was observed in the laboratory that at temperatures below 15 C the fish remained burrowed in the substrate.

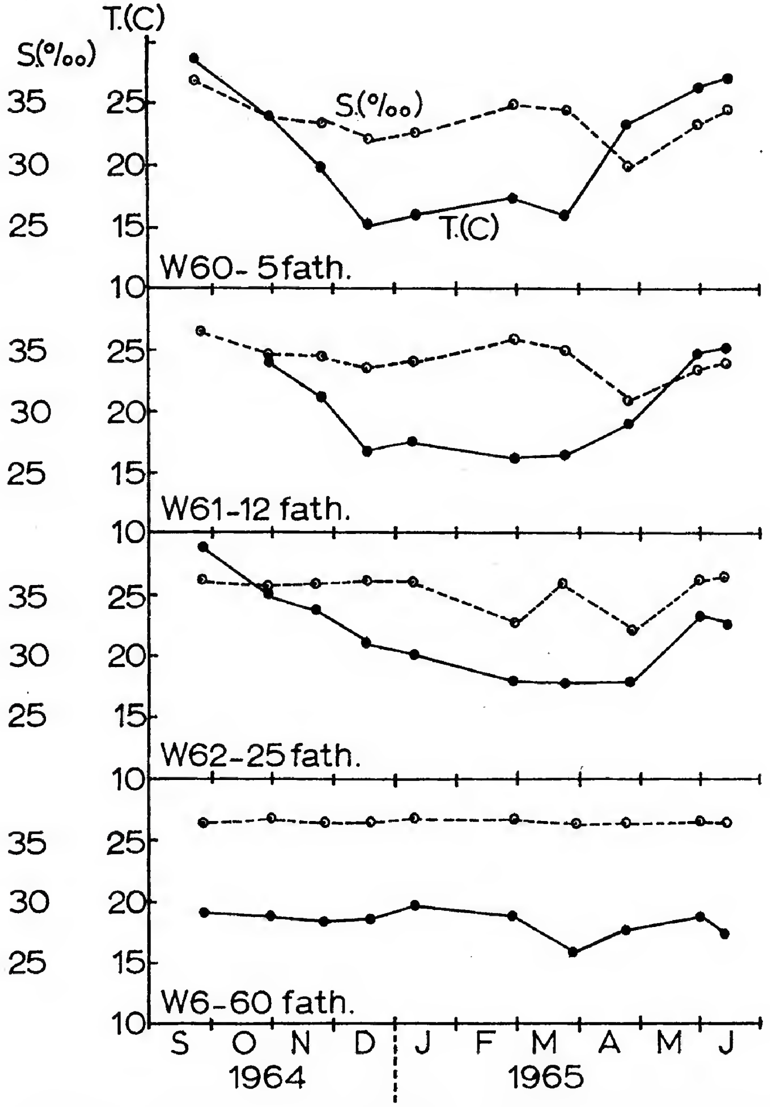


FIG. 13. Bottom temperatures and salinities at different depths in the Gulf of Mexico off Port Mansfield (5–25 fathoms) Sept. 1964 to Sept. 1965. Station W 6 (60 fathoms), was the deepest station and was located off Galveston.

Most spawning occurs in the spring in the bays and in the fall in the Gulf, at times when temperatures approach 25 C. The bay summer spawning, which is of relatively minor importance, occurs at 29–30 C.

Midshipmen are bottom dwellers, burrowing in the substrate during daylight and normally emerging at night to feed. Catch data indicate that the population is most dense over mud bottoms. Depths were similar at bay stations 1 and 3 but the bottom at station 1 was composed of shell and sand and at station 3 of mud. The catch per unit effort was 24.9 at station 3 and 13.5 at station 1. In the Gulf the catch per hour on predominantly sand bottoms (less than 10 fathoms—18 m) was 2.5 while on predominantly mud bottoms (between 11 and 20 fathoms—19–37 m) it was 17.4.

Bottom preference was investigated in the laboratory. Aquaria were supplied with standing, aerated sea water at room temperature in which salinity was controlled between 34.4 and 35.0 ppt. Two aquaria were used, each was 46 × 46 × 153 cm. The substrate in each was one-half Gulf beach sand (about 35 dm^2 of the bottom) and one-half mud from Aransas Bay station 2; substrate depth was 10 cm. The sand-mud ends were reversed in the two tanks.

One tank received eight adults and two juveniles and the other nine adults and two juveniles. In each tank one adult died before the start of the experiment.

Using natural daylength in the first part of the experiment the fish were observed emerging from the substrate at dusk and burrowing into it at dawn. These observations are not presented here as it appeared that the use of a flashlight beam altered the behavior of the fish. When a dim far-red light was used during the night it gave enough illumination to make the outline of the fish visible, but even it affected their behavior. Finally, burrowing was observed by placing the fish on an artificial photoperiod of four hours day and four hours night. When the lights came on suddenly, the fish usually burrowed immediately in whatever substrate was beneath them. In seven instances when they did not, they cruised over the bottom, touching it with their pelvic fins and then entered the mud substrate. Probing of the substrate with a glass rod one-half hour after the light came on indicated that those fish which had initially burrowed in the sand had moved into the mud portion of the tank. The summed results of probing were fifty-eight burrowed in mud and two in sand after one-half hour light.

Midshipmen left trails on the substrate either by swimming close to the bottom or by their burrows collapsing. On mud and adjacent sand, trails were dense, but further away from the sand-mud border they were fewer and there were none at the far end of the sand portion.

It appears then, that although midshipmen did burrow in sand, they seemed to show a preference for mud. This is also reflected in the size of the catches, the largest of which were made over mud bottoms.

In the bays the association of capture success with greater depth indicates that midshipmen avoid very shallow waters (Fig. 14), Aransas Bay station 2 was in the deepest part of that Bay. Pullen (1962) and Parker (1965) reported midshipmen most commonly in the ship channel of upper Galveston Bay suggesting depth as an important factor. They also reported them on oyster reefs but I consider such occurrences to be a seasonal phenomenon.

In the Gulf, midshipmen are most common between 10 and 30 fathoms (18–55 m) (Fig. 14). Compton (1961; 1962) reported them to be most common in the Gulf between 3–10 fathoms (5.5–18.3 m) in 1961 and between 11–15 fathoms (20.1–27.4 m) in 1962. In neither case were his samples very large. Gunter (1958) and McFarland (1963) did not locate midshipmen in the surf zone, but apparently they collected during daylight hours when the midshipmen would be burrowed.

The distribution of midshipmen is affected by a complex of temperature, salinity, bottom type, depth and other unmeasured factors; however, within the tolerances of the species it is most common on mud bottoms and at moderate depths (3–55 m) where sudden external environmental changes are buffered by depth and/or soft mud bottoms.

ASSOCIATED SPECIES

A moderate number of species of both invertebrates and fish were commonly taken in the trawl catch with midshipmen. In the Gulf the fish that are found with midshipmen are listed in Hildebrand (1954a; 1954b) and Miller (1965). *Anchoa* was the most common small fish in Miller's analysis, but Hildebrand used too coarse a mesh to collect anchovies. In the bays the most common species taken with midshipmen are *Anchoa mitchelli* and *Micropogon undulatus* (Hoese *et al.* 1967).

In the Gulf the most commonly trawled invertebrates taken are listed in Hildebrand (1954a; 1954b). In the bays the most commonly taken invertebrates are given in Hoese *et al.* (1967). Penaeid shrimps, Callinectid crabs, *Squilla* and coelenterates were most often taken.

Net selection in both bay and Gulf trawls eliminated from these data the larger fish that could outswim the gear and smaller fish and invertebrates which would pass through the meshes. No bottom plankton hauls were taken, therefore the micro-fauna cannot be included.

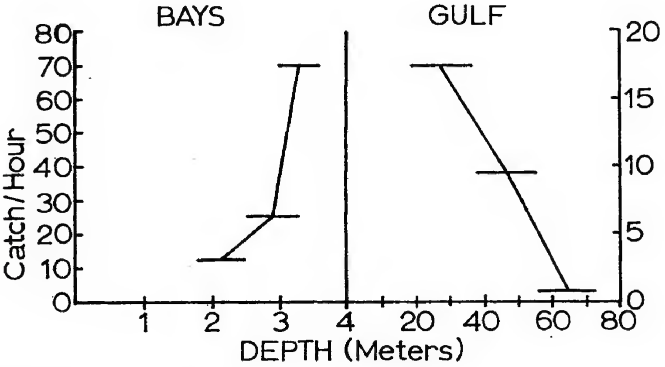


FIG. 14. Depth distribution of midshipmen in the Gulf and bays are shown by catch per hour at various depths in stations with mud bottoms. The bay catches were from a trawl with ¾″ stretch mesh and ¼″ liner and Gulf catches were from a trawl with 2″ stretch mesh. The bars indicate the ranges in depth. Catch per hour averages are based on the whole years data, all months included.

Copeland (1965) lists the most common fish and invertebrates taken by the tide trap in Aransas Pass. Similar gear selection occurs here.

MIGRATION

Movement of midshipmen was detected by various methods including tide trap collections, observations of sexual stages of adults, size of young and population changes in the bays and Gulf. Movement may be divided into three classes; spawning migrations, seasonal movement and other movements.

Spring-spawning midshipmen exhibit the most obvious migrations (Fig. 15). Most ripening adults move from the Gulf through Aransas Pass in February and March, with a few coming in during April. A few bay spawners reside all year in the bays. Those that will spawn in the bays in the summer enter the bays from May to August as ripening adults. The young hatch in the bays and enter the Gulf between early May and late October, with the peak migration in June and July. Along with these young are some spawned adults. A few young remain in the bays all year. Those that spawn in the Gulf, by-in-large, remain there all year; however, a few enter the bays in the fall as juveniles and leave in the sum-

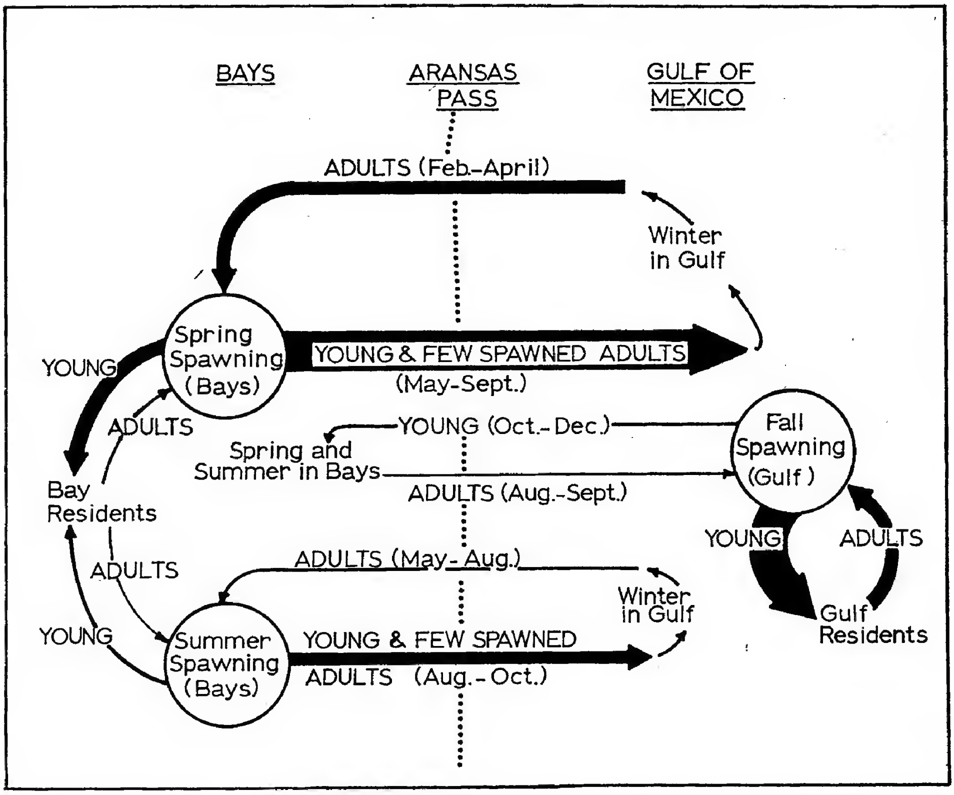


FIG. 15. Schematic representation of spawning migrations of the midshipmen around Port Aransas. Thickness of arrows indicate relative numbers. Bays indicate Aransas, Copano and Corpus Christi Bays.

mer (August and September) as ripening adults. There is no doubt straying of some individuals between the various spawning stocks as there is overlap in the spring and summer bay spawning, and there is little time difference between the summer bay peak and fall Gulf peak (Fig. 16). Spawning migrations for Gulf spawners, if they exist, were not noted.

Seasonal movements in response to seasonal environmental changes also exist. Temperature is probably the most important factor here. Springer (1957) states that midshipmen are absent from bays in winter, and I have found that there is an offshore movement in winter, but it was not very marked. Certainly, some fish remain in bays all winter. Most fish in the bays leave, as young, for the Gulf during the summer. There is a slight trend for more fish to be caught in deeper water in the Gulf during the winter. The four coldest months are December through March (Figs. 12 and 13), during which the trawls obtained 3.7 fish per hour at night from 12–18 fathoms and 4.7 fish per hour at night from 20–25 fathoms despite the greater night catch per hour during the other seasons in the shallower area (18.6 vs 14.6). In general, there are very few fish in the shallow Gulf (3–6 fathoms, 5.5 to 11.0 m) from October through January. In March, the greatest Gulf densities were at 3–6 fathoms (5.5 to 11.0 m); however, this is due to migrating adults heading for the bays. There are a few midshipmen in the shallow water from May to September, but these may also be migrating through the area. The bottom in this area is sandy and, as previously mentioned, not preferred by midshipmen. Only about seven hours of trawling was done in water deeper than 30 fathoms (54.8 m), but during that time only one midshipman was caught.

The other class of movement may be termed wandering. This movement is most obvious in Aransas Pass and here it is primarily caused by tidal currents. Fish that are up in the water feeding at night appeared to move up and down the Pass with the direction of the currents. Fish foraging for food also appeared to move, perhaps following, for short distances, schools of larval fish or groups of

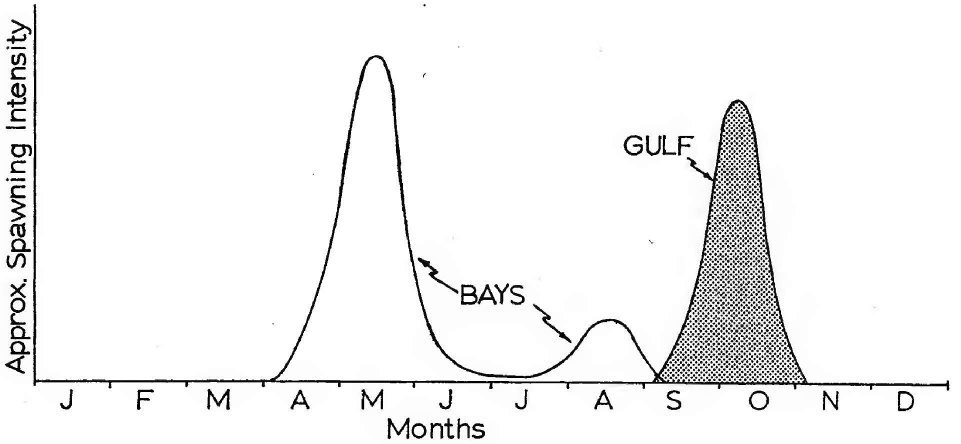


Fig. 16. Annual spawning intensity of bay and Gulf midshipmen. 1. Intensity is a qualitative estimate from collecting time, location, catch, sexual condition and age.

other foods items. Movement may also occur in response to stimuli other than food; *viz.*, bottom configuration.

SEASONAL ABUNDANCES

Bay midshipman populations are large in summer and fall months and small in late winter and early spring for two reasons. Most bay young are spawned in the spring and mortality reduces their numbers during the following year. Moreover, many bay-spawned fish spend the cold months in the Gulf, further reducing the apparent bay population size in winter. The sudden decrease in catch rates in August and September probably reflect such migration. The low winter catch rates in the bays are accentuated by those fish that stay burrowed at night. However, the low catch in the bay during winter was considered indicative of a true decline in abundance of the fish and not because of night burrowing, because temperatures were often high enough to prevent night burrowing and tide trap collections showed a migration out of the bays. As mentioned earlier, the midshipman increase in February and March in the shallow Gulf, at the expense of the population in deeper areas, was due to migrating fish headed for the bays. The October-November peaks in the deeper Gulf resulted from the fall Gulf spawning and the immigration of bay fish into the Gulf. The Gulf resident populations are expected to be highest in the late fall and gradually reduce in numbers until the next fall. The small young were not taken in the large, coarse-meshed trawl available for Gulf collecting.

BEHAVIOR

SPAWNING BEHAVIOR

The northern midshipman *Porichthys notatus* Girard, of the Pacific coast of North America exhibits parental care in guarding its eggs (Greene 1899; Hubbs 1920; Arora 1948). Females leave the nest area immediately after spawning and males stay and guard the eggs. MacGinitie (1935) reported that females guarded the eggs in *P. notatus* but Arora (1948) claimed this was an error in identifying the sex of the fish. Zim and Shoemaker (1956) stated that the Atlantic midshipman male also guards the eggs in a manner similar to that of the toadfish (*Opsanus*). I have no direct evidence of the male guarding the nest in the Atlantic midshipman but there is indirect evidence of the male performing parental care. *Porichthys pososissimus* females produce very few, very large eggs in comparison with most other warm marine fish. Similar numbers and sizes of eggs are reported for other batrachoids which are known to exhibit parental care. Midshipmen appear to spawn in the only areas in the bays, other than the grass flats, where conditions are ideal for nest building; *i.e.*, on sunken shell and oyster reefs. During the inward migration through Aransas Pass, both ripening males and females are encountered. However, in the deeper parts of the bays only females are found indicating that males go directly to the spawning areas. Later, only spawned females are found there, suggesting that they had spawned with the males at some unknown locality and the absence of males indicates that they remained to guard the eggs. These facts suggest parental care being carried out by the male of *P.*

porosissimus. Later in the summer, a few spawned males were collected, the time interval between capture of spent females and males is likely to reflect the time of parental care. The age distribution in the catch indicates that most fish of both sexes die after spawning and it is likely that the reason there are few spent males captured is that most die near the nest as a result of their parental duties. While many spawned females leave the nest after spawning, their condition is uniformly poor; spent females leaving the bays were also in poor condition, indicating that they would not live long, a circumstance supported by the capture of a single 1 + female. Apparently the males that survive to leave the spawning area have a relatively high survival probability.

DIURNAL BURROWING

Midshipmen are nocturnal animals burrowing in the substrate during daylight. This is shown in both laboratory experiments and field data. The trawl catch per hour was higher during the night in nine of twelve seasonal comparisons (Fig. 17). On one occasion the catch was equal and on two occasions the day catch was higher due to one very large catch. The percentage of the trawls in which midshipmen were taken was highest during night hours in eleven of twelve seasonal comparisons. Combining all seasons, the night catches were five and 2.4 times the day catches in the bays and Gulf respectively and the percentage of trawls catching midshipmen was 2.8 times as frequent at night as in the daytime in the Gulf and 1.8 times as frequent in the bays.

In shallow bays where daylight is likely to reach the bottom on all days, diurnal burrowing can be expected to be most marked. It is likely that little light reaches the bottom in the deeper Gulf when the water is turbid. In such circumstances higher daytime catches in deeper water might be expected in a light-avoiding species.

During the four diel trawl sequences catch and light were noted (Fig. 18). In all instances the day catch was very low in comparison with the night catches. It appears that there is a diurnal rhythm in the midshipman which includes burrowing during daylight.

In the laboratory, fish were placed in aquaria with a suitable substrate. Photoperiod was natural daylength (approximately 12 hours light, 12 hours dark), and burrowing and emerging times were noted. This was done by turing on the lights at varying times over several days. It was found that the fish emerged from the substrate approximately 45 minutes after sunset, at which time it was dark. They burrowed approximately one hour to 45 minutes before sunrise, with the very first indications of the dawn. Midshipmen were photonegative, burrowing in response to an extremely small amount of visible light. In an experiment in which a far-red light bulb was used, the light was insufficient to read on the photometer being used (less than 1 microampere), but the fish burrowed. White, yellow, green and blue lights were also tested (wave length from approximately 4000–7800Å), and in all cases fish reacted by burrowing immediately.

Tests were also conducted to see if burrowing was done solely in response to light, or if it would continue in a normal fashion with unusual photoperiods. The normal day-night was reversed, also photoperiods were shortened to three, 4-hour

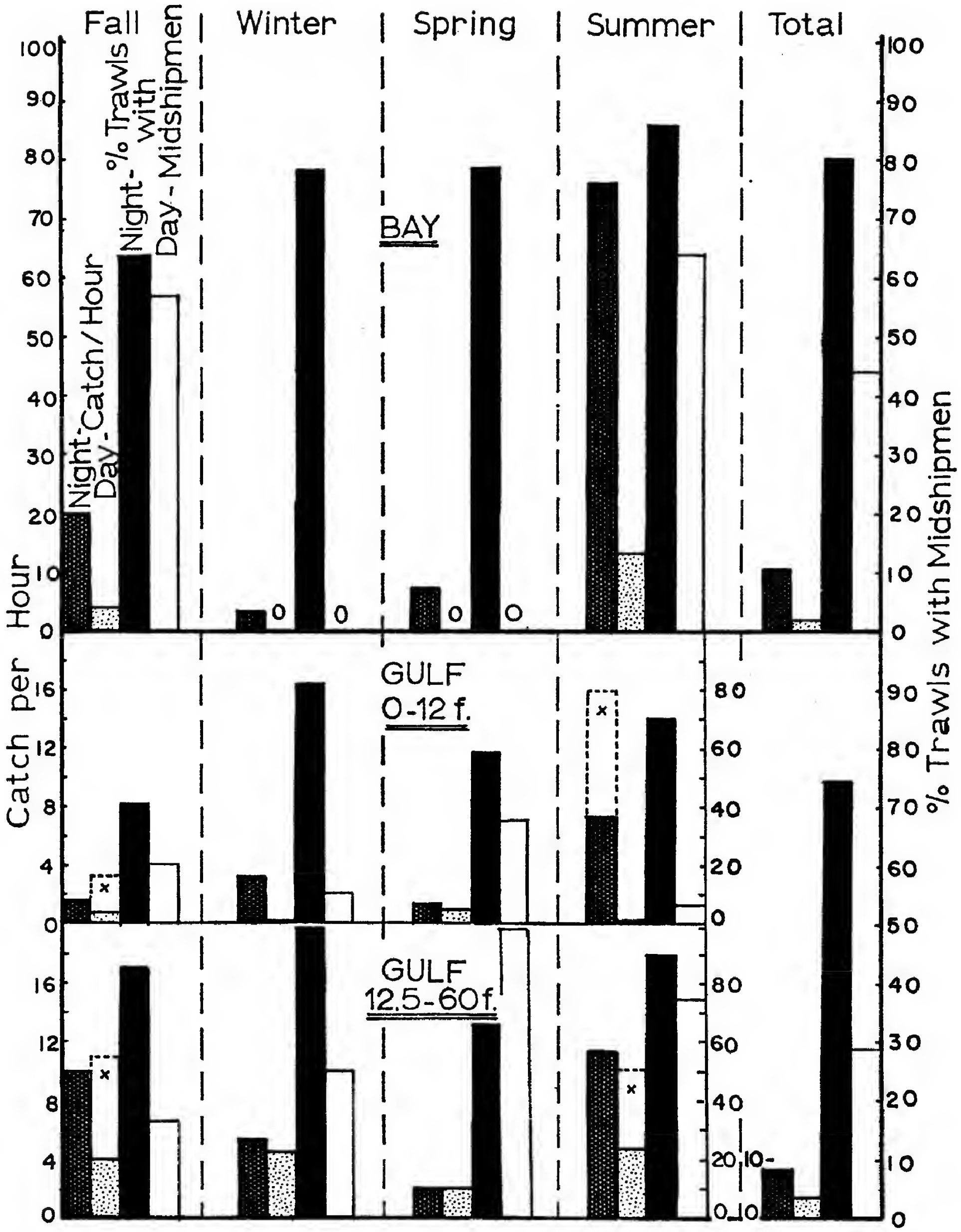


FIG. 17. Catch per hour in numbers of fish and percent of trawls catching midshipmen by night and day in the bays and Gulf. The total columns include all bay and Gulf catches. Dark stipple indicates catch/hour at night. Light stipple indicates catch/hour during daylight. Black bar indicates trawls catching midshipmen at night. Blank bars indicate trawls catching midshipmen during daylight. + and broken line indicate averages with one very high catch, lower part of bar excludes this catch.

day-night cycles in 24 hours. The fish reacted to the change by staying burrowed for up to one week, although most fish changed their behavior in two to four days. After the period of adjustment to the artificial light conditions midshipmen burrowed during the light periods and emerged in dark periods, whenever these occurred.

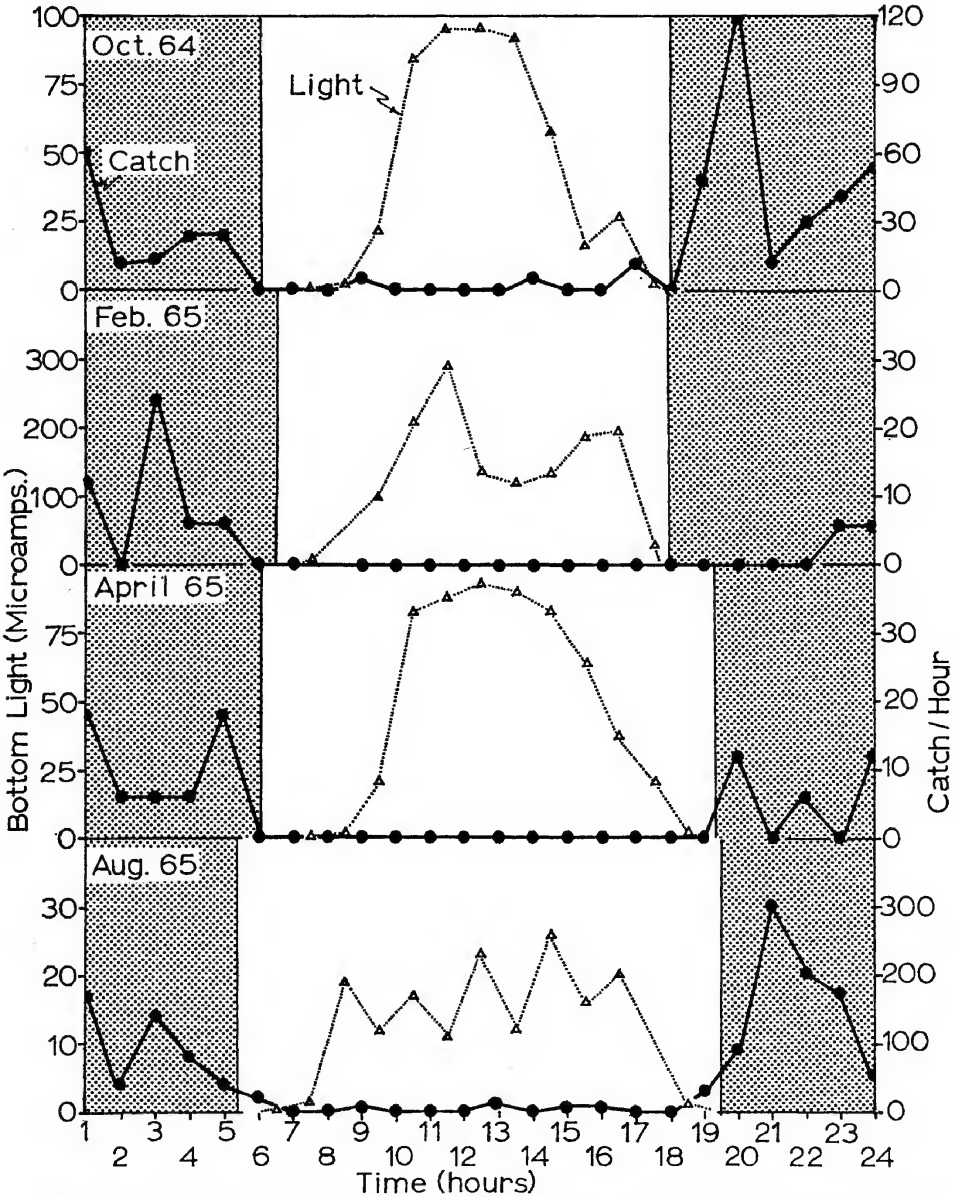


FIG. 18. Bottom light and catch per hour of midshipmen over a 24 hour period at Aransas Bay station 2 in various months of the year. Stippled areas are hours of darkness, light areas, daytime.

SEASONAL BURROWING

When water temperature in the laboratory fell below 15 C many of the fish remained burrowed both day and night. During August an experiment was designed to see what effect winter temperature and photoperiod might have on their diurnal burrowing. Four aquaria, each with five fish, were used. One was kept on normal summer daylength (14 hours light) and temperature (25–30 C), another on summer daylength and winter temperature (16–17 C), a third had a winter daylength (8 hours) and summer temperature and the fourth had both winter temperature and daylength. Observation was started after a twelve day acclimation period. The following results were obtained:

> Summer daylength, summer temperature—fish maintained normal diel rhythm;
> Summer daylength, winter temperature—normal diel activity;
> Winter daylength, summer temperature—fish died, time unknown, no activity noted;
> Winter daylength, winter temperature—fish remained burrowed, all were found alive at the end of the experiment (8 days).

It appears that cool temperature over a three-week period was in itself not sufficient to cause night burrowing but the combination of both short daylength and cool temperature caused submergence for 24 hours each day. The reason for fish death in the short photoperiod, high temperature tank is not known.

The effect of a sudden temperature drop was investigated. Fish were left on normal daylength and the temperature was lowered suddenly from 20 C to 15 C by the addition of ice sealed in plastic bags. The tank used had previously been the long day, winter temperature tank. It was allowed to warm to 20 C, held for two days, during which the fish exhibited normal activity. When the temperature was suddenly dropped 5 C by the addition of ice sealed in plastic bags, the fish remained burrowed at night. Those in the control tank at 20 C under similar conditions without the sudden drop in temperature continued normal diurnal burrowing. It appears then, that a sudden drop in temperature can cause fish to remain burrowed at night. Such conditions periodically occur in bay environments during winter.

When salinity was reduced suddenly from 34.8 ppt to 23.8 ppt by the addition of distilled water, there was no immediate discernible effect upon the activity of the fish.

It is concluded that the fish may not emerge from the substrate at night because of a combination of short photoperiod and cold temperature or a sudden reduction in temperature. Burrowing probably helps to avoid sudden temperature changes since in the bays the mud is somewhat warmer than the water on cold days (Fig. 11). Night burrowing during cold "Northers" might lower the catch in bays during winter.

BURROWING TECHNIQUES

Midshipman can completely disappear from view in three seconds in a mud substrate. When burrowing, the animal lies on the bottom moving its long anal fin from side to side, opening a trough into which the fish settles; the small pelvic fins move in a paddle-like fore-and-aft motion hollowing out a hole in the substrate into which the head sinks. Movement of the large pectoral fins, operculae and branchiostegals clear the mud from around the head. Burrowing is more rapid on the posterior two-thirds of the fish and it goes down first. When the fish is almost completely buried the dorsal fin moves from side to side covering up the back while the head is covered by mud caving in around it. When the burrow is shallow, the fish is slightly inclined so that the tail is deeper than the head, and the eyes are usually extended above the mud surface. The only part of the fish that is visible above the mud are the eyes and sometimes a small part of the dorsal surface of the head. In an aquarium three fish were seen burrowed next to the glass wall. All the deep burrowed ones had a pocket of water around the head, perhaps because the burrow was against the glass. No opercular movement was noted in those fish burrowed against the glass and the manner of breathing under the mud is unknown; perhaps oxygen metabolism is minimal when the fish is burrowed. Fish appeared to be capable of moving through mud. On occasions a fish would be noted to burrow in one area of an aquarium and emerge from another.

TOXIC OPERCULAR SPINE

A description of the opercular spine can be found in a previous section, its connection with bioluminescence will be discussed in the next section. The effects of skin puncture by the spine on humans is severe, even though of short duration. Initially there is a warm sensation where the puncture occurred followed almost immediately (if stuck in the palm of the hand) by considerable pain in the hand and forearm, moving rapidly up the arm to the shoulder. The pain in the arm and shoulder lasts about 15 minutes then subsides during the next 15 minutes. After one-half hour the only remaining pain is caused by the puncture itself. Similar results were felt by three people who were punctured.

It was decided to test the effect of the spine on other fish. Twelve pinfish (*Lagodon rhomboides*), all of similar size (91–111 mm), were used. This fish has a black spot posterior and dorsal to the edge of the operculum. Four fish were left untouched, four were speared in the center of the spot with a steriilzed laboratory probe and four fish were speared in the center of the spot with the spine of a live midshipman. The fish that were not harmed and those pierced with the probe appeared to have no unusual reaction throughout the experiment. Those pierced with the midshipman spine showed immediate signs of stress, lying on their side, gasping and swimming head down. They remained disoriented for approximately 15 minutes and then they began to recover. Twenty minutes after being pierced all had righted themselves, although their reactions appeared to be slow. By the end of 30 minutes the fish appeared recovered; 7 hours later they seemed normal and the experiment was terminated.

The time duration of apparent effect is similar both in *Lagodon* and humans.

BIOLUMINESCENCE

The midshipman is one of the few shallow water bioluminescent fish. Early work on the photophores in midshipman was conducted by Greene (1899) and

Greene and Greene (1924). More recently, Nicol (1957) has shown neural connections to the photophores in *P. myriaster* and concluded they are under the control of the autonomic nervous system. Midshipmen have been stimulated to show their photopores by severe treatment with electrical stimulation (Nicol 1957), sodium hydroxide (Shoemaker 1957) and formaldehyde and adrenaline (Greene 1899; Greene and Greene 1924; Nicol 1957).

Porichthys notatus uses its photophores during courtship display (Crane 1965). Luminiscence in *P. porosissimus* is probably used similarly, but I have not observed it. Springer (1957) noted that the pattern of the photophores in *P. porosissimus* is similar to ctenophores and postulates that the fish uses bioluminescence in mimicry of these comb jellies. I hesitate to accept this theory as the patterns referred to are on the ventral surface of the midshipman, which lives on the bottom, and thus is not visible to most potential predators.

In addition to its probable use in courtship, bioluminescence in *P. porosissimus* appears to be used to warn predators of the midshipmen's toxic opercular spines.

In one experiment a midshipman was lowered into a school of large feeding weakfish, *Cynocion nebulosus*, during daylight when the photopores could be expected to be hardly visible. Other species lowered in the same way as controls included small croaker (*Micropogon undulatus*), spot (*Leiostomus Xanthurus*) and brown shrimp (*Penaeus aztecus*). The controls were eaten immediately. The midshipman was hit by the predators approximately twenty times but was invariably spat out quickly. At the end of the experiment the midshipman, although somewhat toothed marked, was still alive.

A second predator experiment was conducted at night in an aquarium 46 × 46 × 153 cm. It was divided into two parts, one 51 cm long and the other 102 cm long. The predator, a 13-inch grouper (*Parepinephelus* sp.) which had been starved for 24 hours, was placed in the 102 cm part of the tank. A midshipman was placed in the other part six hours before observations were made. A plastic screen and an opaque barrier divided the two parts of the tank. All observations took place after dark. First the tank with barriers in place was observed for 30 minutes on each of two consecutive nights and no luminescence was observed. On both nights, after this observation, the opaque block was removed and with the screen left in place the fish were observed for 15 minutes. On the first night the grouper appeared to be aware of the presence of the midshipman for it immediately swam to the screen, but the midshipman did not flash. On the next night the grouper behaved in a similar manner, but this time it bumped the screen with its snout, and the midshipman flashed faintly once. Later each night the screen was removed and the grouper roamed around the tank. On the first night it approached the midshipman 5 times, and the midshipman flashed 3 of the 5; on the second night the grouper approached the midshipman 3 times, and it in turn flashed 3 times. The grouper also appeared to attack (rapid approach) once and upon seeing the flash of the midshipman immediately retired. Live control fish, croaker (*Micropogon undulatus*), cyprinodont (*Cyprinodon variegatus*) and brown shrimp (*Penaeus aztecus*), were added on both nights immediately after the observation period and were eaten by the grouper within two seconds.

All of the photopores were never visible at any one time. Usually those along the sides (lateral) were visible and twice seemed to be pulsing. Non-pulsing flashes were extremely brief. In approximately 100 hours of observation, no midshipmen were observed to flash in aquaria in the absence of predators. Under natural conditions no observations have been reported of *Porichthys* "flashing" except during spawning.

Hildebrand (*pers com*) has reported to me that trigger fish (Balistidae) will attack, tear apart and eat midshipmen. Trigger fish are one of the few fishes with an extremely hard leathery skin. It would be difficult to penetrate its skin with a spine and this may be why it is a predator of midshipmen. It is noteworthy that Hildebrand reported that the trigger fish tears its prey apart thereby removing the likelihood of being stung inside its mouth. Jordan (1886) found a midshipman in the stomach of the snapper *Lutjanus aya* (=*L. campechanus*). Moseley (1966), in his study of the food of *Lutjanus aya*, found no midshipman in any of the stomachs he examined.

It is concluded that the small nocturnal Atlantic midshipman with a toxic opercular spine is avoided by predators and that bioluminescence is probably used as a warning device to potential predators.

SPAWNING AND FECUNDITY

Evidence of the time and place of spawning was obtained from the numbers of ripe and spent adults and from the very young midshipmen caught in the experimental fishing. Three annual peaks of spawning activity were found in the population around Port Aransas. In Aransas and Copano Bays spawning peaks occur in late April through May and again in early August, with less activity between peaks. The April-May peak is by far the largest (Fig. 16). In the Gulf, spawning occurs off Port Aransas and Port Mansfield in the latter part of September and the beginning of October. This peak appears to be second in amplitude, larger than the August peak in the bays but smaller than the April-May peak; however, magnitude of spawning is difficult to compare between bays and the Gulf because of differences in the area involved.

Spawning in the bays probably occurs on sunken reefs and shoals between 1.5 and 2 m in depth. I collected in the deeper areas of the bays (1.8–3.6 m) and never found spawning adults; I also collected in the shallower areas (0–1 m) and again did not find spawning adults. While such evidence is questionable, I assume that spawning occurs in the intermediate depths. Pullen (1962) and Parker (1965) found midshipmen on oyster reefs. It is likely that sunken reefs are the main spawning areas in the bays. This is in agreement with the described spawning behavioral patterns for *Porichthys notatus* in the Pacific (Hubbs 1920; Arora 1948) and the Atlantic midshipman elsewhere (Zim and Shoemaker 1956). In the Gulf, collections were made during the spawning period at 7.5, 12, 15 and 25 fathoms (13.7, 21.9, 27.4 and 45.7 m). At all depths except 15 fathoms (27.4 m) the proportion of ripe or spawned individuals to non ripe individuals was less than 15%, whereas at 15 fathoms (27.4 m) it was greater than 80%.

AGE AND SIZE OF SPAWNING ADULTS

Most midshipmen spawn and die when they are approximately one year old. A moderate percentage (approximately 20%) of the males live to be two years

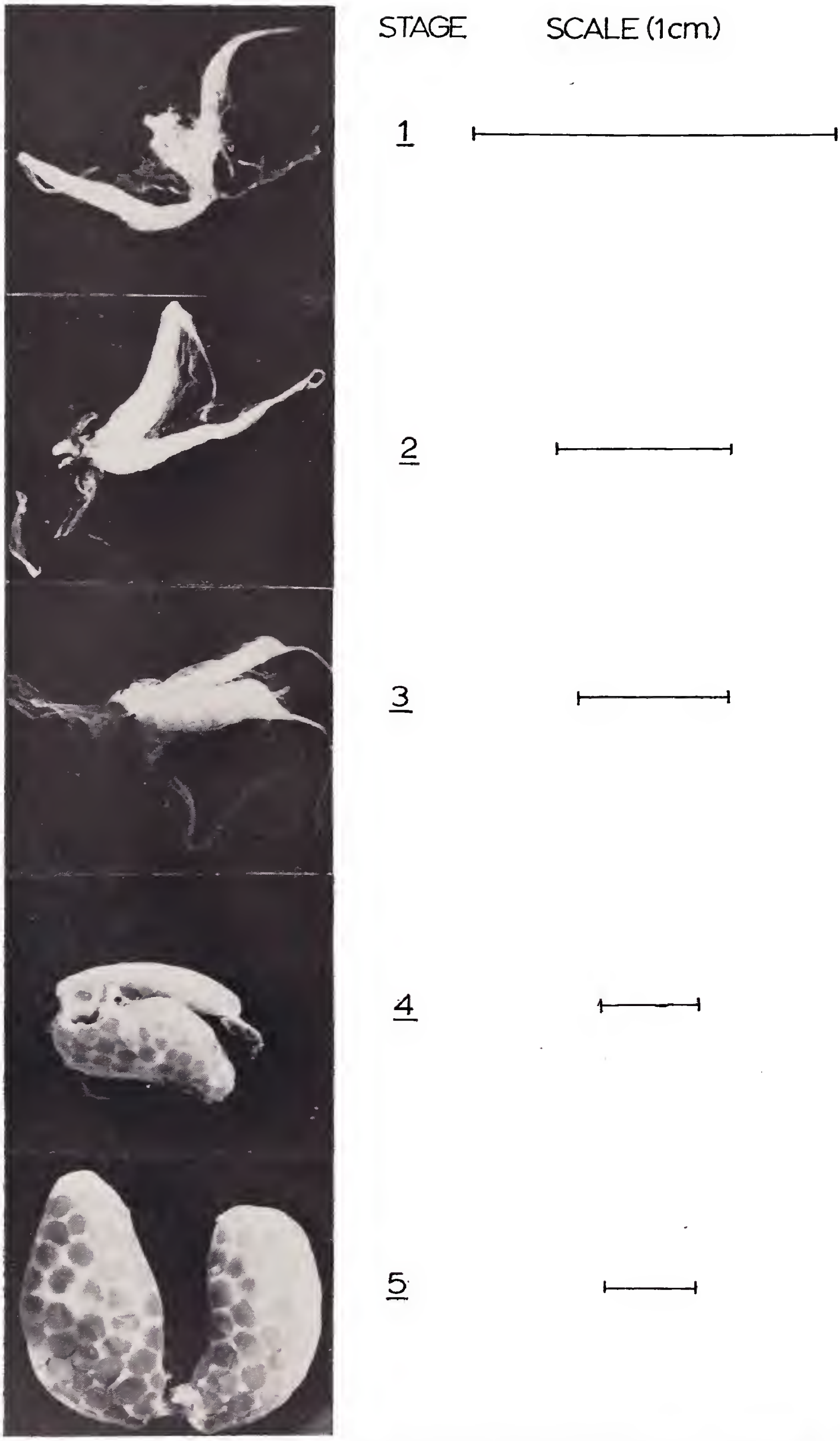


Fig. 19. Maturation stages of the female gonad. Stages correspond to Table 3. Note the very small white eggs between the larger eggs in stages 4 and 5. Scale is 1 cm.

old. No marking experiments were conducted to determine whether the males spawned twice or did not spawn until their second year. Only one female (less than 0.5% of those sampled) was more than one year old and she was ripening to spawn at age two; one male was over two years old.

Spawning females were longer than 92 mm standard length and ripe males were longer than 80 mm. Few individuals of either sex were mature smaller than 100 mm. Maximum sizes observed were 139 mm for females and 186 mm for males, but few fish were found larger than 125 mm.

MATURITY AND FECUNDITY

Stages of maturity were arbitrarily designated, based on gonad volume for males and egg appearance and diameter for females. Egg diameter was measured using an ocular micrometer; egg counts were total counts except in ripening females where there were eggs of two size groups (one very small and one large) and only the larger were counted (Fig. 19 and 20, Table 3 and 4).

Fish that were hatched in May and June would reach stage two between November and March, development from stage two on is quite rapid with the fish going through the last four stages in a period of 3 to 7 months.

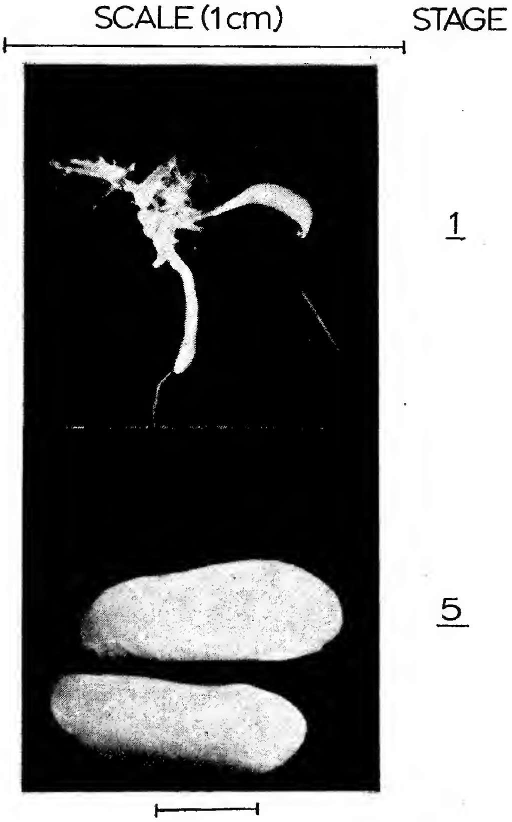


Fig. 20. Maturation stages 1 and 5 of the male gonad. Stages correspond to Table 4. Scale is 1 cm.

TABLE 3

Description of the female gonad from preserved specimens. The notation on visibility of the nucleus is based on examination under a dissecting microscope with an incandescent microscope lamp

Stage no.	Diameter of eggs (range and means in mm.)	Total gonad volume (ml.)	Egg description	Age of fish[1] (months)	Standard length of fish (mm.)
0	0—0.20	<0.05	very small. no visible nucleus	0—5	0— 83
1	0.10—0.60 x̄=0.30	0.05—0.20	small. larger cells with visible nucleus	3—8	62—109 x̄= 83
2	0.10—1.5 x̄=0.60	0.05—0.40	small. most cells except largest ones with visible nucleus	5—9	77—120 x̄=100
3	0.20—2.50 x̄=1.0	0.2 —0.9	maturing. smaller cells only with visible nucleus	6—12	91—119 x̄=106
4	0.8 —3.5 x̄=2.6	0.7 —4.9	ripening. no visible nucleus	9—24 usually 11 or 12	92—139 x̄=110
5	2.8—>4.0 x̄=3.5	2.7 —5.7	ripe. no visible nucleus	9—24 usually 11 or 12	92—139 x̄=112
6	variable	variable	spawned. attretic eggs may be present, membrane loose	over 9	92—139

[1] Determined by length frequencies and otoliths.

TABLE 4

Male gonad analysis. Maturation stages, testes volume and the length of the fish found within the various stages

Stage no.	Gonad description	Testes volume (mm.)	Minimum length of possible second spawners[1]	Length of fish (range in mm.)
0	little or none	<0.02	86	0—135
1	some	0.02—0.05	86	64—167
2	maturing	0.06—0.50	86	84—157
3	further maturing	0.51—1.00	124	80—169
4	ripening	1.01—2.00	124	93—156
5	ripe	>2.00	124	88—144

[1] It is not known if all the larger males have previously spawned; however, from the appearance of the testes in the larger fish in stages 0 to 2, it is likely that many have.

The number of eggs was found to be related to the size of the fish. The logarithm of egg number appeared to be linearly related to standard length of fish (Fig. 21). Such a relationship determined by examination of 67 ripe females may be described by the equation;

$$\text{Log } F = 0.00968\,L + 1.0647,$$

where F = egg number, and
L = standard length.

The correlation coefficient (r) of these data was 0.836.

Midshipmen have a low number of large eggs, an unusual occurrance for a warm water marine fish. The mean egg number was 140.5, with an average diameter for ripe eggs of 3.5 mm. Spawning fish average 110 mm standard length.

Attempts to hatch midshipmen in the laboratory were not successful; however, a continuous series of juveniles from 13 mm up was captured. Time of capture and the presence of a small amount of yolk sac indicates the smallest fish were recently hatched. It is probable that they become free-swimming between 12 and 15 mm in length. Smallest specimens indicate that midshipmen do not undergo any notable metamorphosis outside the egg except in the development of the photophore pattern.

In summary, spawning of midshipmen on the Texas coast may be divided into three periods; a spring peak and a summer peak in bays and a fall peak in the Gulf, where spawning probably occurs at 15 fathoms (27.4 m). The temperature was approaching 25 C in the spring (bay) and fall (Gulf) spawning times and was above 25 C during the summer peak. Most fish are one year old when they spawn and usually between 100–120 mm standard length. Most die after spawning; approximately 20% of the males spawn in their second year but rarely do females. Larger females have more eggs than smaller females; midshipmen females average 110 mm standard length and have approximately 140 eggs. Young

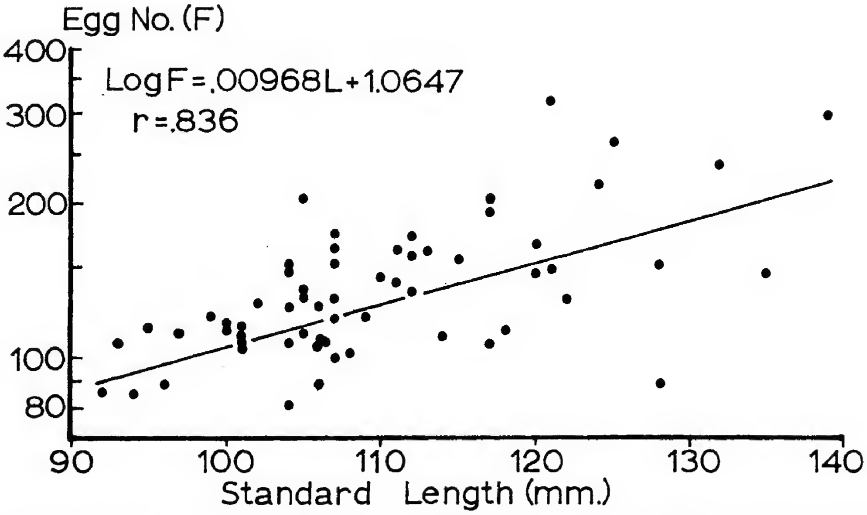


Fig. 21. Midshipmen egg number (log scale) plotted against standard length. Log F = .00968L + 1.0647.

do not undergo any overt metamorphosis after hatching and are free-swimming by the time they are 12–15 mm.

LENGTH-WEIGHT RELATIONSHIP

During the study 2013 midshipmen were weighed and measured after preservation. They were sorted into size groups and 50 fish from each group were selected at random for calculation of a length-weight curve. A length loss of about 2–3% occurred in the first 48 hours of preservation in 4 % formaldehyde diluted with sea water. Parker (1963) and others have found losses in length of 2–4% and loss in weight of 4–10% in 4% formaldehyde and sea water solutions; most of which occurs in the first 24 hours of preservation.

The length-weight relationships (Fig. 22) determined from 500 midshipmen, ranging from 13 to 169 mm, may be described by the formulae;

$$W = 8.85\ (10^{-6})\ L^{3.139} \text{ or}$$

$$\text{Log } W = 4.947\text{–}10 + 3.139 \text{ Log } L,$$

where W = weight after preservation, and

L = length after preservation.

The regression fits the formula $W = aL^b$ given in Ricker (1958), with the power term $b = 3.139$ indicating nearly isometric growth. Some variation in the length-weight relation existed among fish between 95 and 125 mm standard length because this was the size at which females spawned. Those which had spawned were relatively lighter than those which were ripe. My sample included about 50% of each group, and the relationship in the length range between 95 and 125 mm represents an average for both ripe and spawned females along with males.

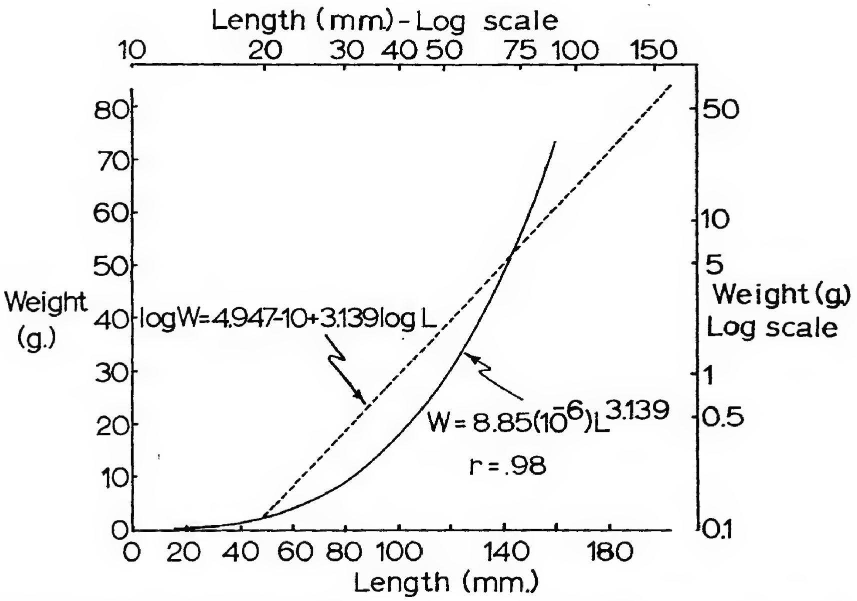


Fig. 22. Length-weight relationship of midshipmen from around Port Aransas. Dashed line is the log-log regression, solid line is the arithmetic relationship, n = 500.

All fish over 139 mm were males. Below 125 mm the sexes did not differ in the length-weight relationship.

GROWTH RATE

As previously noted most midshipmen live only one year. Ages were determined by use of otoliths, and the results were verified with age determinations by size frequency (Fig. 23). Newly hatched fish did not have discernible otolith rings. Fish larger than 45 mm almost always have a fall ring and may add another ring in the spring. The young hatched in the bays in April-May 1964 grew at essentially the same rate as the young of April-May 1965, and these data were combined in Figure 24. Mean lengths for the monthly collections increased from May through February except October-November, where the samples were small. Growth of midshipmen is essentially linear over their life, except when they are very young and during final maturation and spawning.

The spring spawning stock provided the majority of the young fish collected and therefore was used for the examination of growth. Young from other spawning periods were excluded from the analysis. The smallest fish, 30 mm or less, could easily be allocated to a spawning period by size frequency analysis. Larger individuals were more difficult to allocate, but use of collection locality, otoliths and fin ray counts, permitted most fish to be placed in a spawning period. The allocations were less reliable with increases in age. Lengths at various ages were also subject to bias by net selection. The trawl used in the bays tended to select for smaller individuals (under 80 mm), and that used in the Gulf caught mostly larger fish (over 85 mm). Therefore, when fish entered the Gulf in the fall, net selection might have tended to increase the apparent growth rate.

Fish older than 13 months were not included in Figure 24 because only a few specimens were obtained. It appears, however, that midshipmen do continue to grow at a linear rate, although somewhat slower than their first year. Too few fish were available from the summer and fall spawning periods for a determination of their growth curve.

The relationship between otolith length and fish length appears linear except for fish less than 20 mm (Fig. 25).

FOOD HABITS AND FEEDING

FEEDING

Midshipmen's diet, anatomy and behavior indicate two methods of feeding; filter and raptorial. I have never observed the midshipman feeding, but they lie just below the mud water interface with the dorsally placed, telescopic eyes just above the mud surface. Probably when a food item passes close to the hidden midshipman it darts out, catches its prey and then retires into the mud. Other species (*e.g., Uranoscopus*) have been noted to feed in this manner (Nicol 1960). The manner of burrowing, with the eyes above the mud surface, has been observed in midshipmen kept in aquaria; also, the fish has been seen to dart very rapidly for short distances. The second method of feeding, which the midshipman probably utilizes, is filter feeding for microplankton crustacea.

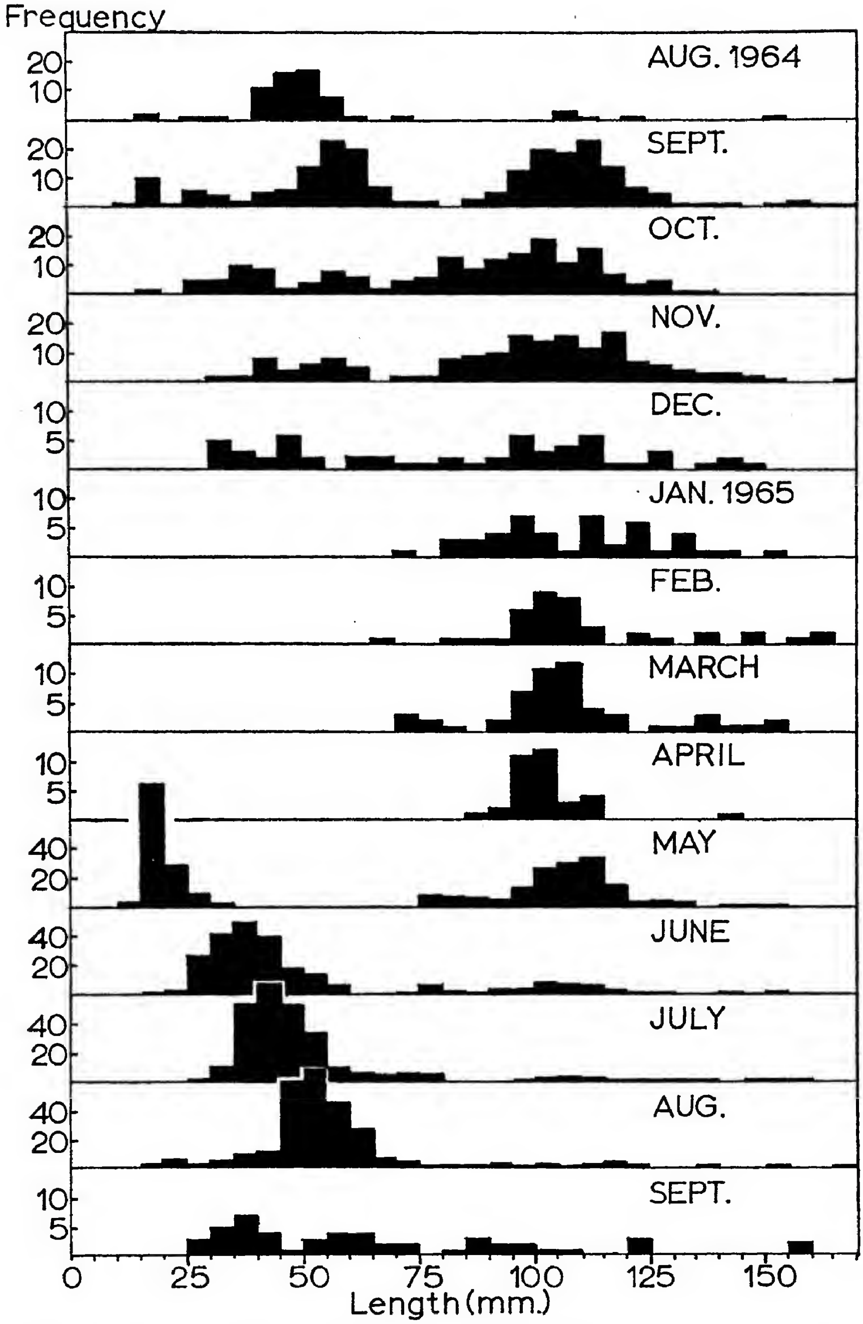


Fig. 23. Monthly length-frequency distribution of 2087 midshipmen caught by trawl in Aransas and Copano Bays and the Gulf of Mexico. Note the changes in the frequency scale.

The gill rakers in *Porichthys porosissimus* are quite long and numerous (approximately 15 on the lower half of the first arch). When viewed through the mouth the gill structures appear to form a coarse sieve. Small mysidacea and ostracodes are probably captured by filter feeding.

FOOD

Stomachs from at least 25 bay fish and 25 Gulf fish were examined each month, except in the few cases when fewer fish were captured. The area and date of col-

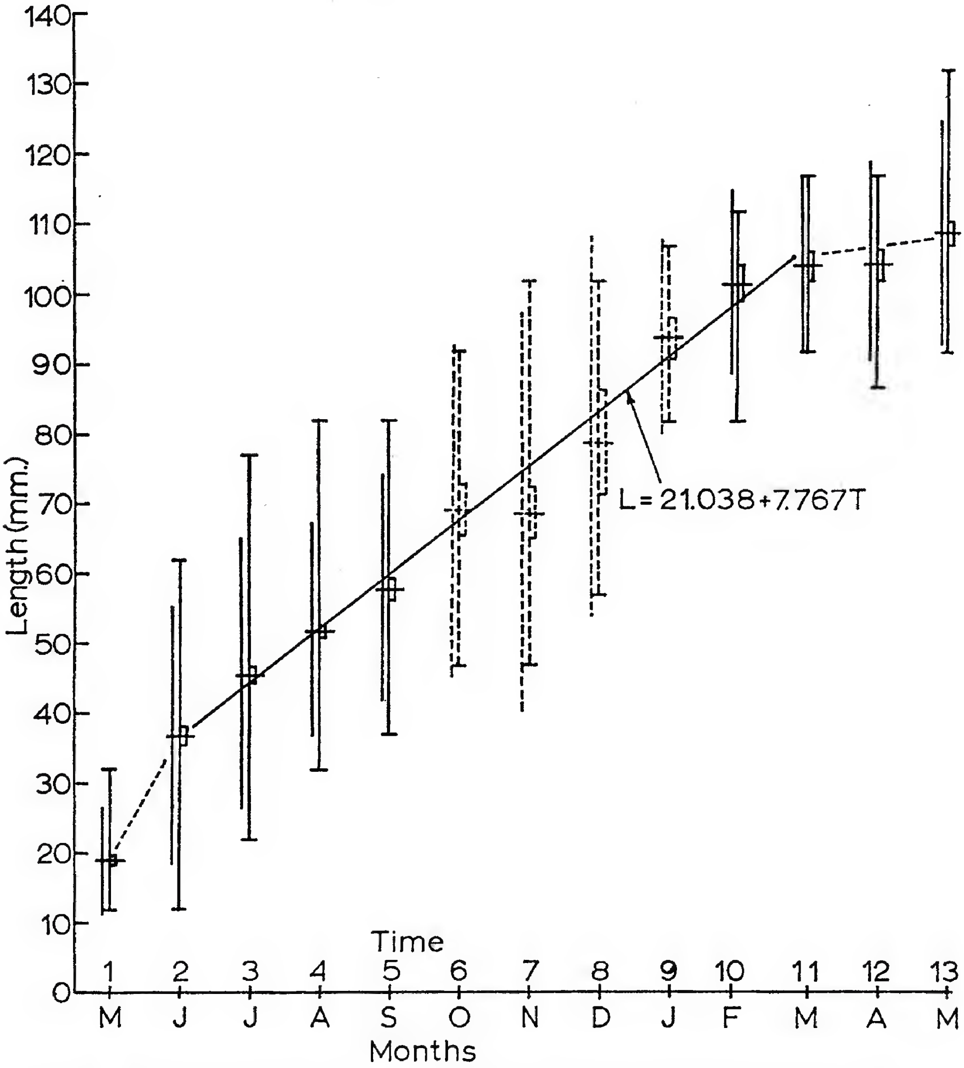


FIG. 24. Growth curve of midshipmen, determined specimens aged 2 months to 11 months. Months (6–9) shown by broken lines were not included in the calculation of the line because samples were small over this period. In the formula, L indicates standard length in mm and T is time in months. The long vertical center line is the range in lengths, the short horizontal line is the mean, two standard deviations on each side of the mean are indicated by the line to the left of the range and two standard errors on each side of the mean by the box to the right.

lection, size of fish, sex, gonadal condition, food items, food position in the gut, and tooth sexual dimorphism were noted for each fish. Volumes of food organisms were only occasionally noted for small food particles, but were measured for all larger articles; total volume was measured for full stomachs. The gut was arbitrarily divided into three areas: anterior, which included the esophagus and stomach; mid-gut (small intestine); and posterior gut, which included the intestine behind the last bend (the "large intestine" and rectum).

Food organisms were usually identified to genus and only occasionally to species. Much of the food items were larval crustacean bottom plankton, which were usually identified to order, suborder or family.

Items found in the 588 stomachs containing food are shown in Table 5. The table shows all food from all fish regardless of area in which captured, sex or size. It is evident that midshipmen feed mostly on small crustacea and fish. The latter comprises the most important energy source in midshipmen larger than 50 mm standard length (it takes many mysidacea to equal one fish).

Diet in terms of numbers (not volume) by region, size and sex is shown in Figure 26. The diet of fish from the Gulf and bays was essentially similar, but those in the Pass appeared to eat more fish, squid and polychaetes than those in the other areas. Gulf fish ate more Amphipoda than both those in the bays and Pass; the latter being the lowest. Fish from the bays ate more Mysidacea than

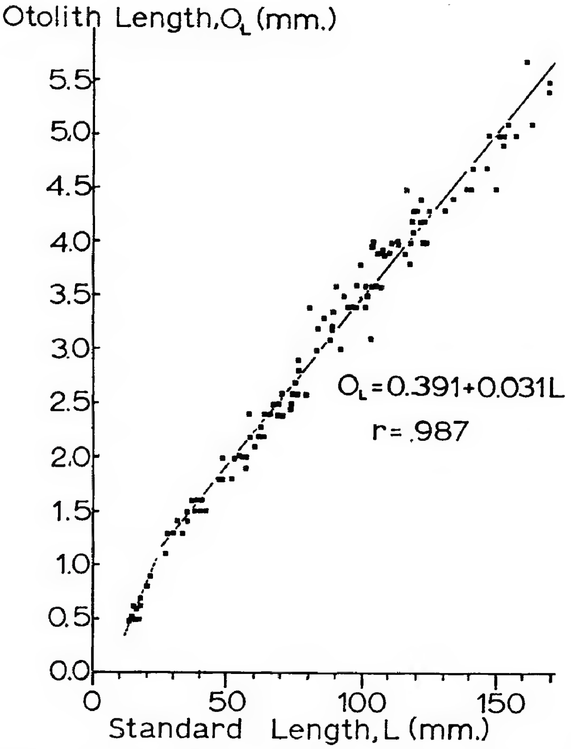


FIG. 25. Relationship between otolith length (O_L) and fish standard length (L). Line for smallest fish was fitted by eye. n = 117.

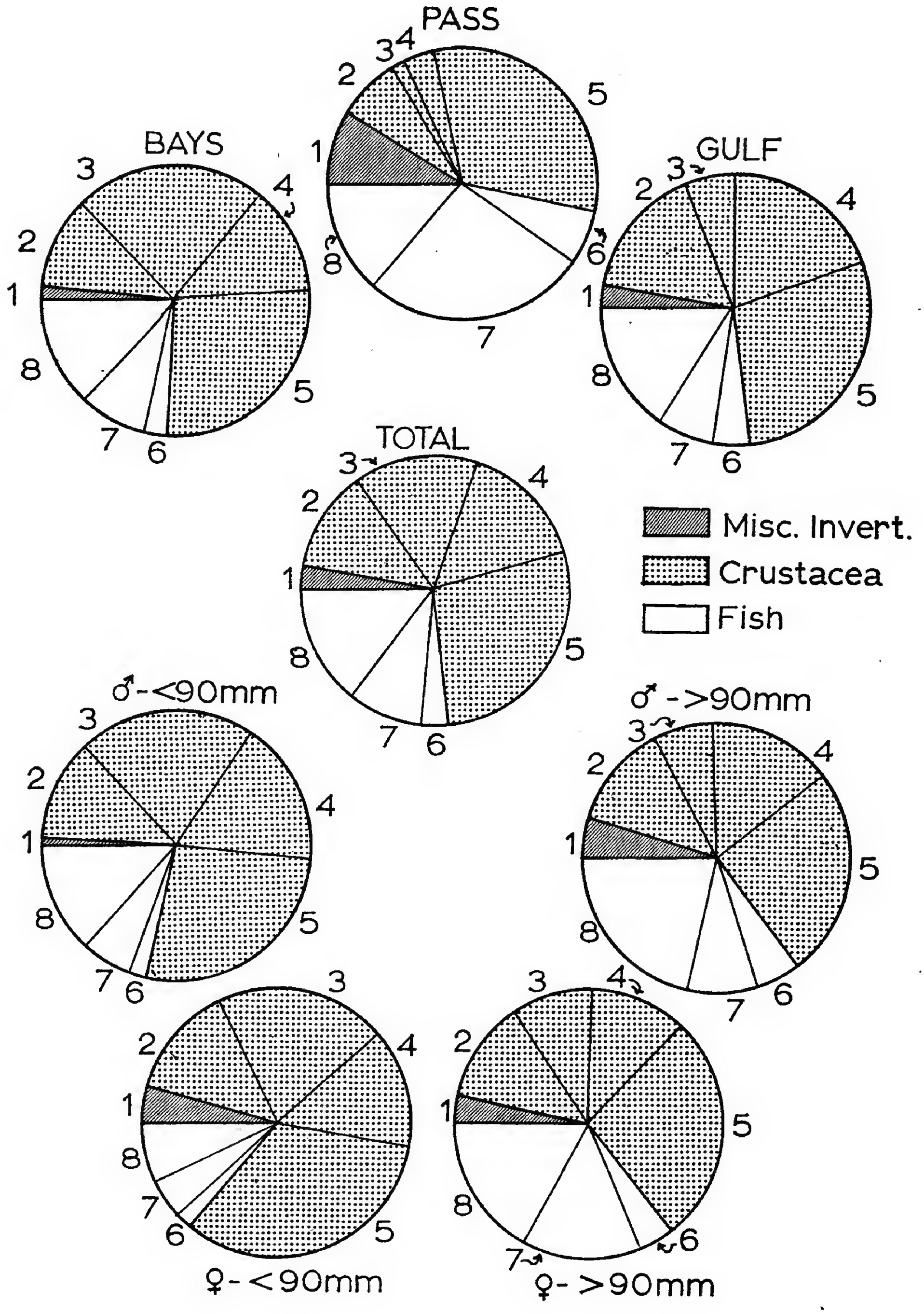
TABLE 5

Food of *Porichthys porosissimus* from the Texas coast. Numbers in brackets indicate the percentage occurrance of the food item in the sample of 588. Most unidentified groups contain partly digested items similar to those identified; these partly digested remains probably include a larger proportion of infrequently eaten food items because of my greater familiarity with remains of common foods

1. Very Common Items (9–28%)
   - Crustacea—unidentified larvae to juvenile (24%)
     - Mysidacea—unidentified (10%)
       - Mysidae—*Mysidopsis bigelowi* (19%)
     - Amphipoda—unidentified (13%)
       - Suborder Hyperidae (9%)
     - Macrura—unidentified (13%)
   - Vertebrata—Pisces—unidentified larvae to juvenile (28%)
     - —Engraulidae—*Anchoa* mostly
       - *A. mitchelli* larval to juvenile (18%)
2. Common Items (5–9%)
   - Crustacea—Cumacea—Distylidae (6%)
     - Amphipoda—Suborder Gammaridae (7%)
     - Macrura—Penaeidae unidentified larvae to juvenile (6%)
     - Brachyura—unidentified larval to juvenile (7%)
3. Uncommon Items (1–4%)
   - Annelida—Polycheta (2%)
   - Mollusca—Cephalopda—Loliginidae—*Lolliguncula* (2%)
   - Crustacea—Ostracoda (2%)
     - Copepoda (2%)
     - Stomatopoda—Squillidae—*Squilla* post larval (1%)
     - Macrura—Penaeidae—*Penaeus* larvae to juvenile (2%)
       - *Trachypenaeus* larvae to juvenile (1%)
       - *Parapenaeus* larvae (1%)
     - Sergestidae unidentified larvae to juvenile (2%)
       - *Lucifer* larvae to juvenile (2%)
     - Caridae—unidentified larvae to juvenile (1%)
       - Anomura—Paguridae larvae to juvenile (1%)
     - Vertebrata—Pisces—Clupeidae—*Harengula* larvae to juvenile (4%)
       - Sciaenidae—*Micropogon undulatus* post larvae to juvenile (2%)
4. Rare Items (all less than 1%)
   - Mollusca—Pelecypods—Doncidae—*Donax*
     - Pteropods
   - Crustacea—Mysidacea—Mysidae—*Gastrosaccus*
       - *Mysidum*
     - Leptostraca—*Nebalia*
     - Cumacea—Bodotriidae
     - Isopoda—unidentified
       - —Cirolanidae—*Cirolina*
       - —Cymothoidae
     - Macrura—Penaeidae—*Xiphipenaeus* juvenile
       - —*Sicyonia* larvae to juvenile
       - —Scyllaridae larvae
       - —Palaemonidae—*Periclimones* juvenile
       - —Rhynchocinidae—*Rhynchocinetes*
     - Anomura unidentified
     - Brachyura—Portunidae—unidentified
       - —*Callinectes* larvae to juvenile
       - —*Ovalipes* juvenile
     - unidentified crustacea eggs
   - Echinodermata—Crinoidae—Bourgueticrinidae
   - Vertebrata—Pices—Synodontidae—*Synodus* juvenile
     - —Bregmacerotidae—*Bregmacerus* juvenile
     - —Gobidae—*Gobionellus* juvenile

those taken from the Gulf or the Pass; and fish from the Pass contained more *Anchoa* than those from the other areas.

There is little difference in the diet between the sexes. Small fish ate more crustacea and fewer fish than larger midshipmen. There appears to be a relation-



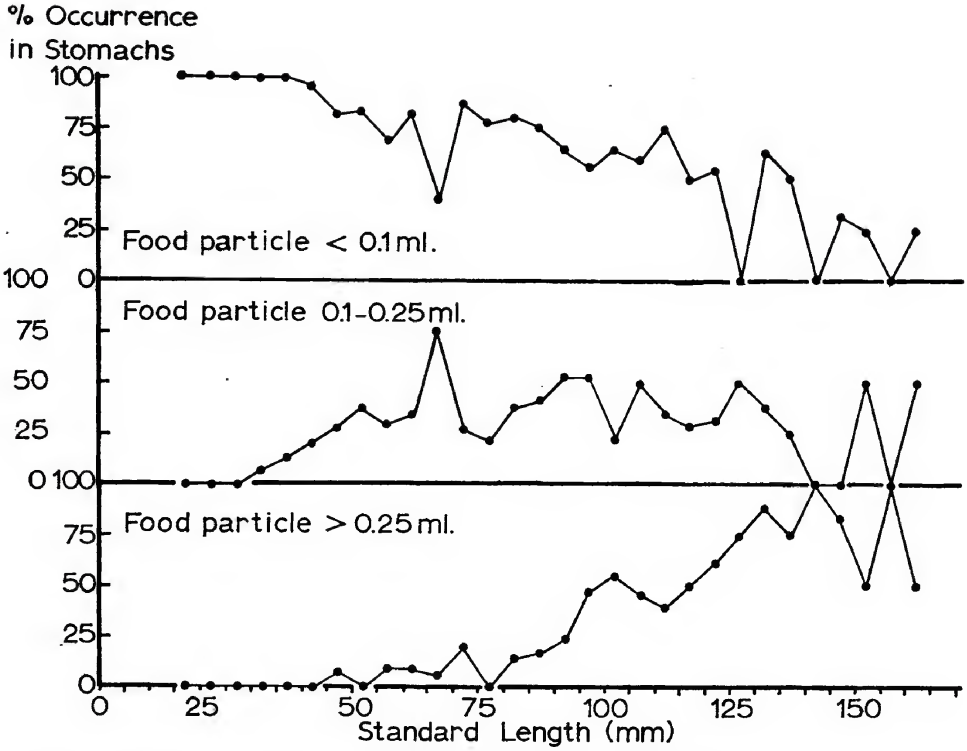


FIG. 27. The relationship between size of food particles and length of fish. Items eaten include mostly planktonic crustacea and larval fish.

ship between food size (of all kinds) and size of fish, with larger fish consuming larger articles of food (Fig. 27). However, some small food items did occur in fish of all sizes.

There were no seasonal trends in food among the very common food items (Category 1 in Table 5). Within the common group (Category 2 in Table 5), Brachyuran larvae appeared to be more prevalent in spring and summer than at other times of the year. Among the minor food items, the following appeared to be seasonal; Sergestidae—spring, Paguridae—summer, *Squilla*—spring and summer, *Micropogon*—winter and early spring and *Harengula*—summer.

Empty stomachs were found in all size ranges, with smaller fish having a lower

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FIG. 26. Proportions of food items in 588 Atlantic midshipmen stomachs stratified by area of capture, sex and size (based on numbers). All fish caught by trawling or tide trap in the study area between July 1964 and October 1965.

1 = miscellaneous invertebrates (mostly Polycheta and Mollusca)
2 = unidentified crustacea
3 = Mysidacea
4 = Amphipoda
5 = other crustacea (Table 5)
6 = other fish (Table 5)
7 = *Anchoa*
8 = unidentified fish

percentage of empty stomachs. Of the fish having a standard length of less than 45 mm approximately 4% had empty stomachs; in the fish above 100 mm, 21% had empty stomachs. In adult bay fish, on their spawning migrations, 40–70% of the stomachs were without food. The spawning adults taken in the Gulf, between 30–40% had stomachs without food. Hubbs (1920) noted that most spawning *Porichthys notatus* had empty stomachs.

The volume of full stomachs increased with length, approximately exponentially. The maximum volume measured in full stomachs from midshipmen less than 50 mm was 0.12 ml, from 50–74 mm it was 0.4 ml, from 75–99 mm it was 0.6 ml, from 100–149 mm it was 2.0 ml, and one 164 mm midshipman had a fully extended stomach which contained 6.2 ml of food.

DIGESTIVE RATE

The position of the food in the alimentary canal was noted to see if it was possible to estimate the time of maximum feeding and the digestive rate. The gut was divided as previously discussed and the results shown in Table 6.

The main feeding period is at night, as 80% of the stomachs with food had some of it in the anterior gut (Table 6). Midshipmen begin feeding upon emergence from the substrate at dusk and the peak feeding occurs between then and midnight. This is followed by a period of nocturnal digestion as many of the individuals are satiated with food. Just before dawn there is another peak of feeding activity before burrowing in the substrate where the midshipmen remains during the daylight hours. The rate of digestion and elimination appears to be somewhat related to feeding rates. Food in the posterior gut of fish taken at dusk or early evening was almost completely digested, suggesting a moderate length of time in the gut. Food in a similar gut position from fish captured between midnight and 0100, with full stomachs, was often partially digested. Hence, the rate of movement of food through the gut seems to be partially related to the amount entering the anterior end.

In summary, it appears that the Atlantic midshipman feeds primarily on small bottom planktonic crustacea and fish, and shows little food selection. There was considerable similarity in diets in both bays and Gulf. Young fish eat smaller food items, thus largely a crustacean diet, while larger fish eat small and large food items, thus a considerable quantity of fish and larger crustacea along with planktonic crustacea. Peaks of feeding activity occur upon emergence from the substrate at dusk and during the early night, and again before dawn; almost no feeding occurs during daylight.

TABLE 6

Approximate time of day and percentages of stomachs with food in various portions of the alimentary tract. Based on all stomachs sample between Nov. 1964–Aug. 1965 inclusive (407)

Light	Anterior gut	Mid gut	Posterior gut
Dawn	55%	73%	27%
Day	18%	54%	90%
Dusk	47%	55%	90%
Night	80%	69%	60%

MACROPARASITES

Few parasites were found living on or in the midshipman. The only parasite that was common was an unidentified nematod. It was usually found in the anterior and mid portions of the intestine. The proportion of fish parasitized was related to the size of the fish (Fig. 28). As fish size is closely related to age, it appears that the proportion of the midshipman population infested by nematodes is a function of age. It might be noted, however, that parasites were found in fewer than one-third of the fish.

Cestodes were found in fewer than 1% of the adults and in no young. They were usually found in the subcutaneous muscle of the body and were easily identified by the presence of external bulges on the skin. Ectoparasites were found only on five fish and were all an Isopod gill parasite belonging to the family Cymothoidae.

SUMMARY

The Atlantic midshipman, *Porichthys porosissimus* (Valenciennes), is a common bottom fish in the Northern Gulf of Mexico and the associated bays. The species lives in shallow water from Virginia south to Argentina. There is considerable variation in color pattern and meristic counts, both throughout the geographic range and within relatively small areas. Such differences were found between midshipmen that spawn in the bays and those that spawn in the Gulf of Mexico around Port Aransas.

The Atlantic midshipman is bioluminescent, having many rows of photophores

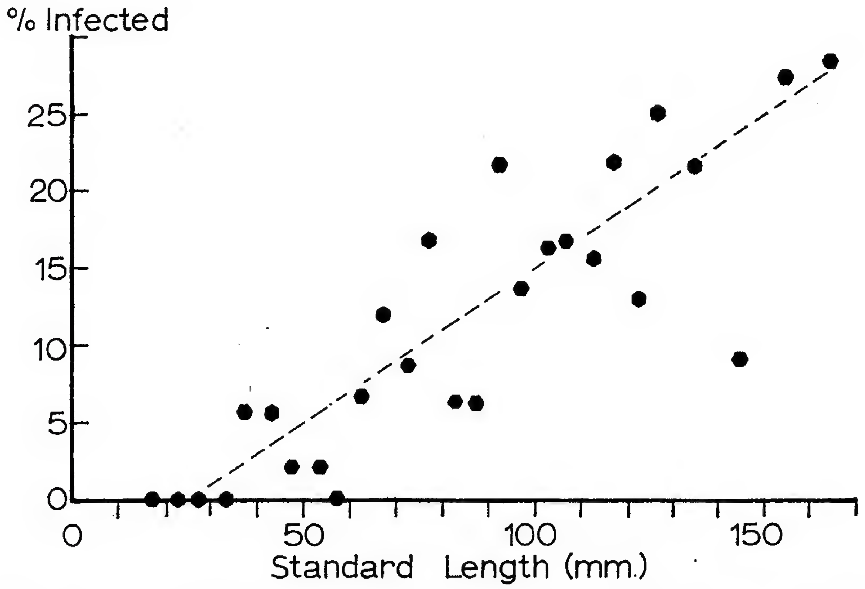

Fig. 28. The relationship between percentage of midshipmen infested with nematodes and standard length of fish. Line fitted by eye. Each point represents the % infested within a 5 mm length group.

on its body and head. It is equipped with a poisonous opercular spine. Experiments indicated that the bioluminescence is useful as a warning device against potential predators. The fish also uses its biological light in courtship displays.

The midshipman is a polyhaline and polythermal animal, but is not common in areas of extremely high or low salinity or temperature. It appears to be most abundant in areas where salinity is between 20–36 ppt, and where temperature is greater than 25 C for at least one season of the year. Midshipmen burrow in the substrate during daylight and appear to show a preference for mud bottom. Although burrowing appears to be normally a photonegative response to light, continuous (24 hours) burrowing was experimentally induced by rapid reduction of water temperature or reduction of both temperature and photoperiod.

There are distinct migrations; many adults migrate into the bays to spawn in early spring and summer. Most offspring leave the bays for the Gulf in the late spring to early fall and return to the bays the next year as adults. Around Port Aransas, midshipmen have two spawning areas and three time periods; the greatest spawning activity is in spring in the bays, the summer bay spawning is less, and the fall Gulf spawning appears to be intermediate.

Most midshipmen spawn and die at the end of their first year, reaching 90 to 135 mm standard length in that year. A few (mostly males) live another year, and have been recorded to a length of 186 mm near Port Aransas. No females were found over 139 mm. Females produce few, but large, eggs (up to 5 mm diameter). The average female is 110 mm long with 140 ova. No major metamorphosis was evident after hatching, and young become free swimming between 12 and 15 mm.

Midshipmen appear to feed almost exclusively on small crustacea and small fish. Amphipods, mysidacea and the fish *Anchoa* were found most abundantly in stomachs. Few macroparasites were found in *Porichthys porosissimus*, a gut nematod being the only one of possible importance.

ACKNOWLEDGMENTS

I am indebted to Drs. B. J. Copeland, Clark Hubbs, W. F. Blair and M. Menaker of the University of Texas, also Drs. D. W. Coble, J. C. MacLeod and Mr. W. J. Christie, Research Branch, Ontario Dept. of Lands and Forests, for their advice, suggestions and critical reading of the manuscript; Dr. D. E. Wohlschlag helped with the statistical analyses; many helped with the field work, especially Dr. H. D. Hoese, Messrs. Frank Moseley, David Steed and William Gillespie; Capt. Herman Moore, skipper of the M/V Lorene; the U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries, Galveston Laboratory, for making possible the Gulf of Mexico collecting trips, especially Messrs. Bruce Kimsey and Robert Temple, program leaders of the Gulf Shrimp Fisheries program; Mr. David Harrington for his assistance in voyage planning and scheduling; Messrs. Harold Brusher and Stewart Law for their help aboard the M/V Gus III; the captains and crew of the M/V Gus III; Mr. Henry Compton who drew the frontispiece; and the many people, too numerous to mention, who helped in other ways. Josephine Lane is thanked for editorial help.

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PHYSIOECOLOGY OF THE GRASS SHRIMP, *PALAEMONETES PUGIO*, IN THE GALVESTON BAY ESTUARINE SYSTEM[1]

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ABSTRACT

The seasonal distribution, growth rate, and reproductive potential of the grass shrimp, *Palaemonetes pugio*, as affected by temperature and salinity were studied in the Galveston Bay estuarine system, by means of a field survey of 13 stations from March, 1963 to February, 1964, and a controlled laboratory experiment.

Grass shrimp appeared throughout the year with greatest abundance in July and October. Abundance was greatest in waters of 10–20 ppt salinity and least in < 1.0 ppt salinity. The abundance of the sexes (males, ovigerous females, and non-ovigerous females) of grass shrimp is related to temperature with the sex ratio (males to all females) approximately 1:1.

Growth to maturity (20–24 mm) takes two to three months during June–September and four to six months during the remainder of the year. No appreciable increase in length or weight could be attributed to salinity. Greatest increase in length and weight attributed to temperature occurred in waters of > 30 C and least in waters of 11C to 14 C. There is a size difference between males (23.5 mm), non-ovigerous females (26.2 mm), and ovigerous females (30.0 mm). Longevity of grass shrimp is about one year.

P. pugio can survive over a wide range of salinities and temperatures with best survival in waters of 4–16 ppt salinity and 18 C to 25 C.

Ovigerous females were collected from March to October with two peak spawning seasons, one in early summer (July) and the other in early fall (October). Water temperatures of approximately 18–20 C are required for reproduction to occur. The largest females (28–42 mm) produce

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[1] Modified from a thesis presented to the graduate college of Texas A&M University for partial fulfillment of the degree of Master of Science, January, 1965.

egg masses in the first spawning peak with gradual replacement by smaller individuals in the second peak. The number of eggs carried by ovigerous females increased in a geometric progression related to length. Number of eggs in an egg mass is a linear function of the female's length.

INTRODUCTION

The grass shrimp, *Palaemonetes pugio* Holthuis, is prominent in the diet of numerous sport fish of the Gulf coast including the channel bass or redfish and the speckled trout (Gunter 1945; Miles 1949; Moody 1950; and Darnell 1958). This study of the grass shrimp was stimulated by the assumed importance of this species in the food web of the Galveston Bay system of the Texas coast. Information is presented here on growth rate, reproductive potential, seasonal occurrence and distribution as affected by temperature and salinity to help clarify the role of *P. pugio* in an estuarine ecosystem.

The detailed physiological and ecological tolerance levels of *P. pugio* have not been studied. The rate of larval development, survival and frequency of molting of *P. pugio* and *P. vulgaris* was shown by Broad (1957). The effect of salinity on the egg size of *P. varians*, *P. mesogenitor* and *P. antennarius* in Europe was described by Solland (1932). Dobkin and Manning (1964) studied the osmoregulation of the two species *Palaemonetes* from Florida. The effect of temperature and salinity on osmoregulation in crustaceans, which included *P. varians* and *P. antennarius*, was given by Lockwood (1962). Panikkar (1941) described the mechanisms of osmoregulation in *P. varians* and *Leander squilla.* The tolerance of *P. vulgaris* to waters of low salinity was given by Nagabhushanam (1961). The physiological reproductive activities of *Palaemonetes* were described by Burkenroad (1947), and Yonge (1955) described egg attachment in the Family Palaemonidae.

Several life history and biology studies have been conducted on fresh water *Palaemonetes* in the United States (Broad and Born 1963; Meehean 1956; and Gebhart 1936). Lofts (1956) and Gurney (1923) studied the general biological requirements and distribution of *P. varians* in Britain salt marshes.

The general ecological habitat of *Palaemonetes pugio*, together with other brackish water species of the same genus, was described by Holthuis (1952). Although no research on the detailed ecological requirements of the estuarine species has been done, *P. pugio* has been included in several ecological surveys made within the geographical range of the species (Gunter 1945; 1950; Miles 1949; Moody 1950; Baldauf 1953; 1954; Reid 1955; Simmons 1957; Darnell 1958; Hildebrand 1958; Chambers and Sparks 1959; Arnold, Wheeler and Baxter 1960; Pullen 1961).

This study was conducted in two phases, a field study and a laboratory experiment.

STATISTICAL PROCEDURES USED FOR BOTH FIELD AND LABORATORY DATA

In the laboratory experiments all sexes of *P. pugio* were pooled for the purpose of performing statistical analyses on their lengths and weights. Data from the field survey were separated into three groups: males, non-ovigerous females and ovigerous females.

Randomized Block Design and Modified Split Plot Design analyses of variance were performed to estimate the effects of time, temperature, salinity and salinity-temperature interactions on the observed variation of length and weight in the field and laboratory. Procedures used for the analyses of variance are those given by Snedecor (1956).

In an analysis of variance test a significant F value does not indicate which mean value differs significantly from another mean value. For this reason Duncan Multiple Range Tests, at the 5% probability level, were conducted to separate significant means. The Duncan range test reduces the possibility of accepting the null hypothesis when that hypothesis is wrong. Procedures for the Duncan Multiple Range Test are given by Snedecor (1956). However, because of unequal numbers in the samples of grass shrimp collected or sampled, a weighted error term was used in computing the confidence ranged values of some of the Duncan Multiple Range Tests (Wood 1965).

FIELD STUDY

The first phase was a survey of populations of *Palaemonetes pugio* in the shore zone of the Galveston Bay estuarine system. Of more than 30,500 specimens of *P. pugio* collected from 11 collecting trips, 9043 were measured and their sex determined.

DESCRIPTION OF AREA AND STATIONS

The studied area in Galveston Bay extended from fresh waters of Upper Trinity Bay to salty waters of Lower Galveston Bay and East Bay (Fig. 1). The perimeter of this area consists primarily of coastal marshes, bayous, beaches, and a major and one minor river outlet. Locations of 13 collecting stations occupied during the study are shown in Figure 1 and described by Wood (1965).

SAMPLING

A pushnet (Allen and Inglis 1958) was used in sampling at shore-zone stations from 23 March 1963 to 28 February 1964. Collections were made monthly by pushing the net in water 6 inches to 2 feet (15.24–40.96 cm) deep for a distance of 65 feet (19.8 m) parallel to the shore line. Initially, a permanent marker was set at each sampling site to insure use of the same area on subsequent trips. A 65-foot (19.8 m) nylon cord attached to the marker was used to determine the length of each tow; therefore, the number of specimens collected per station gave an approximate indication of abundance. Usually two replicate tows about 3 feet apart (1 m) were made at each station. At the end of each collection, air and water temperatures were measured and a water sample was taken 6 to 12 inches (15.2–28.5 cm) beneath the surface for salinity determinations. Samples of grass shrimp were preserved in 10% formalin containing 2% glycerin.

The order of collecting at the stations each month depended largely on weather conditions and the feasibility of covering a given area within a reasonable time.

KINDS OF MEASUREMENTS

Length-Frequency Distribution. In the case of large samples, only 100 grass shrimp were identified, sexed and measured. The remaining specimens were only counted. In collections containing less than 100 shrimp, all specimens were measured and sexed. Total length, the distance from the anterior end of the rostrum to the posterior edge of the telson, each specimen was measured to the nearest 0.5 mm.

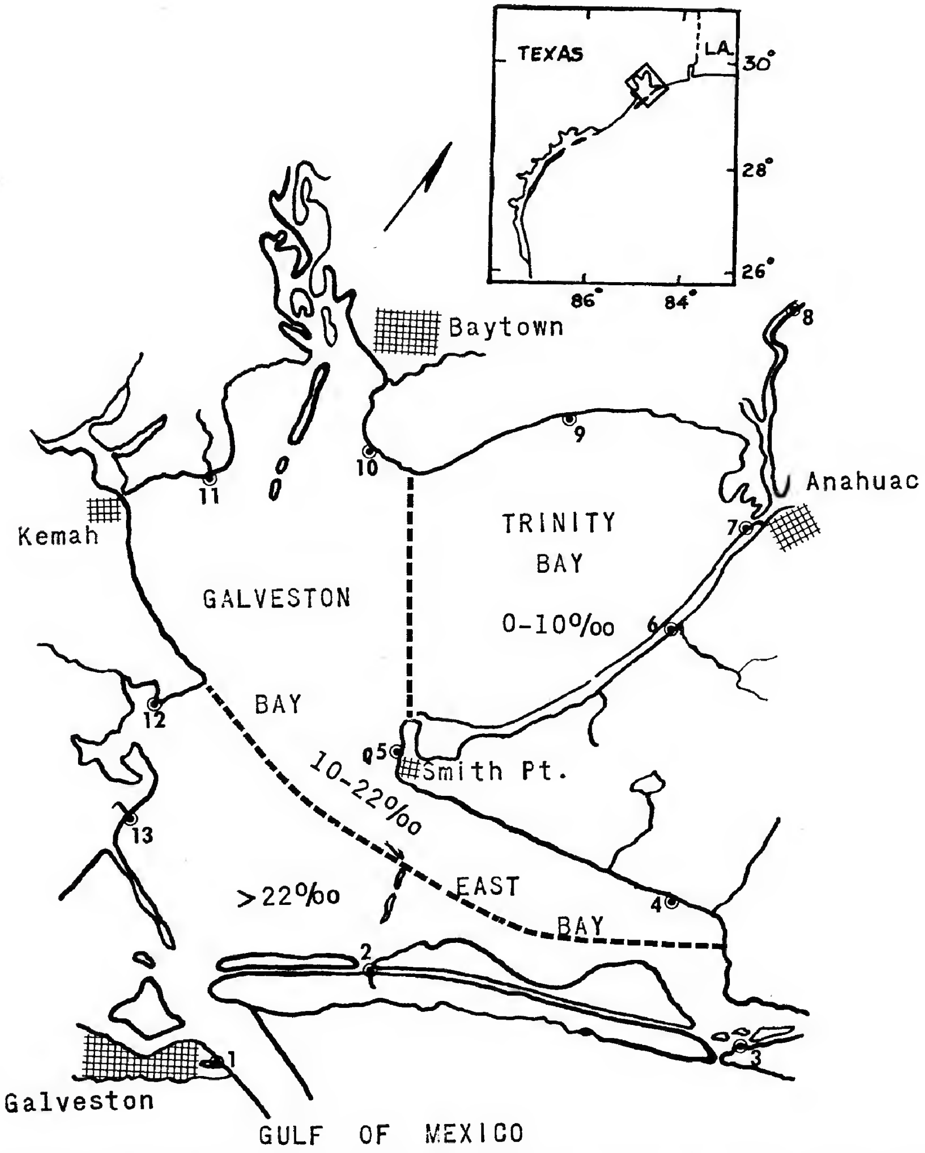

FIG. 1. Salinity regime and collecting stations in the Galvestion Bay system from March, 1963 to February, 1964.

Reproduction Characteristics. The sexes of all measured specimens of *P. pugio* were separated by size differences of the endopodite of the first pleopod previously described by Meehean (1956) for *P. paludosa.* This character was found to be reliable in all shrimp examined. Broad (personal communication) indicated that in *P. kadiakensis* this character is valid in all shrimp having a carapace length in excess of 2.5 mm. Also, in live shrimp, the vasa efferentia of the mature males are usually visible through the carapace.

All females were examined for the presence of an egg mass attached to the ventral abdomen. The greatest length and width of the egg masses were recorded from 36 individuals ranging in total length from 19.5 mm to 41.5 mm. The egg masses were then removed from the individuals and the number of eggs in each mass was counted and recorded with a hand-tally counter. Regression analyses were performed to determine the relationship between the numbers of eggs carried by a female, total length of the female, and the area of the egg pad.

HYDROGRAPHIC DATA

Salinity. The positions of major streams emptying into the bays, the shape of the basins and the position of channels, spoil banks and oyster reefs divide the area roughly into 4 separate but related hydrographic systems. However, in this survey, only 3 ecological areas (based on the average salinity) were found (Fig. 1). These areas contain waters that are oligohaline (<10 ppt), Stations 6, 7, 8 and 9; oligohaline to mesohaline (10–22 ppt), Stations 4, 5, 10 and 11; and metahaline (>22 ppt), Stations 1, 2, 3, 12 and 13. Table 1 gives the actual salinity measurements taken during the study.

Overall salinities of the bay system were above normal during June–January when compared to other yearly data collected by the U.S. Weather Bureau. The only time the salinity returned to a normal condition during June–January was when Hurricane Cindy's path crossed the eastern section of the system. Effects of the storm on the salinity regime lasted for only about one month.

Temperature. Water temperature was considerably influenced during the day by cloudiness, wind velocity and height of waves.

Table 1 gives the water temperatures recorded on each collecting trip. The water temperatures may be classified into four seasonal temperature ranges or groups as follows:

6 to 14 C, December and January;
14 to 22 C, February, March and November;
22 to 30 C, April, September and October; and
30 to 38 C, June, July and August.

RELATIONSHIPS BETWEEN ENVIRONMENTAL CONDITIONS AND THE DISTRIBUTION AND ABUNDANCE OF *Palaemonetes pugio*

Temperature. Numbers of grass shrimp obtained monthly at all stations during March, 1963 to February, 1964 are given in Table 2.

Greater numbers occurred in July and October, the months of peak spawning. Although grass shrimp were less abundant in other months, there were some present during each month of the sampling period. The effects of the low temperatures (Table 1) are indicated by the few specimens taken in December and January. Presumably, the grass shrimp move away from the edge of the shore to deeper water during severe winter temperatures.

The seasonal abundance agrees closely with the findings of Broad and Born (1963), Meehean (1956), Lofts (1956), Gebhart (1936) and Gurney (1923), who show that most of the recruitment of *P. kadiakensis*, *P. exilipes*, *P. paludosa* and *P. varians* occurs in July. The second recruitment of *P. pugio*, which occurs in the fall, is similar to that of *P. paludosa* (Meehean 1956). Meehean's length-frequency studies (Fig. 7, p. 437) show that two spawning periods are distinguishable. These findings are in partial agreement with Gunter (1950), because they show that as the temperature is lowered the grass shrimp move to deeper water.

TABLE 1

Water temperature (C) and salinity measurements (ppt) recorded in the field

	Collection trip										
Station	March I	April II	June III	July IV	Aug. V	Sept. VI	Oct. VII	Nov. VIII	Dec. IX	Jan. X	Feb. XI
1	[a]17.5	26.8	30.5	32.8	30.4	29.4	25.0	16.2	10.0	12.3	14.2
	[b]27.5	25.5	28.1	31.2	33.9	32.0	29.9	29.1	27.4	31.1	29.5
2	14.5	25.4	29.1	29.2	28.0	28.0	24.5	15.2	...	11.5	15.0
	25.2	22.1	24.1	25.1	28.1	24.3	25.3	29.3	...	27.3	25.0
3	17.8	24.8	29.5	30.8	29.8	27.8	24.3	14.9	...	12.4	15.0
	18.3	23.9	21.5	26.1	27.1	21.5	22.1	15.4	...	7.8	13.9
4	17.4	26.2	30.1	31.0	30.4	26.3	24.2	12.5	...	12.0	15.4
	17.5	20.0	22.6	16.5	19.6	21.0	15.0	14.1	...	10.1	...
5	22.0	29.0	30.0	32.1	30.2	26.5	26.1	14.3	...	12.3	14.2
	18.6	19.8	16.7	17.2	21.8	23.2	20.5	21.9	...	14.3	16.3
6	20.5	28.0	31.5	34.3	30.5	28.5	25.1	16.2	...	12.5	15.0
	10.3	13.3	2.3	10.4	11.5	10.6	14.5	20.8	...	16.9	10.3
7	21.5	26.9	31.6	34.5	30.5	28.5	25.7	15.0	...	13.5	16.0
	10.3	3.6	3.6	9.2	10.1	13.0	17.4	20.0	...	17.1	8.1
8	20.5	26.4	31.4	32.4	31.5	29.3	26.7	18.6	6.9	13.5	13.9
	0.4	0.3	0.1	0.9	1.3	2.0	1.1	0.6	0.4	0.2	0.1
9	21.5	26.7	35.0	37.5	33.0	31.5	29.6	18.6	...	12.8	14.0
	8.0	11.5	8.9	9.1	15.6	15.0	14.2	19.6	...	5.7	10.4
10	20.3	27.3	32.8	35.1	32.8	29.5	28.2	17.2	...	12.3	14.0
	11.4	16.5	15.9	15.4	17.0	18.4	23.1	20.9	11.5	14.1	17.0
11	21.0	28.4	30.0	29.9	31.8	29.5	28.9	16.0	10.1	12.0	15.5
	16.1	19.4	18.5	16.4	18.3	24.7	25.9	13.5	11.6	15.4	15.2
12	20.5	28.3	30.7	30.6	32.0	30.1	28.2	17.2	9.8	12.5	15.5
	18.2	22.5	20.3	21.2	27.5	24.5	25.3	21.8	17.9	16.4	16.8
13	23.2	29.2	30.0	30.1	30.0	29.5	28.0	16.7	10.3	13.0	16.0
	16.4	17.8	21.3	28.8	30.0	27.6	27.2	22.0	10.3	22.0	24.5
Water Avg.	19.9	27.2	30.9	32.3	30.8	28.8	26.5	16.1	9.4	12.5	14.9
Salinity Avg.	15.2	16.6	15.7	17.5	20.1	19.8	20.1	19.2	13.2	15.3	15.6

[a] Water temperature.
[b] Salinity.

However, some grass shrimp were taken throughout the year during this study regardless of the temperature.

Salinity. Total numbers of grass shrimp captured at each station for the entire year are shown in Table 2. Greater numbers were obtained at Stations 6 and 7 in Trinity Bay and at Stations 11, 12 and 13 in Galveston Bay and few were taken at Station 8 or in fresh water. The number of *Palaemonetes* at Stations 6, 7, 9 and 10 increased abruptly in July, whereas they decreased in July at Stations 1, 2, 3 and 13. The numbers caught at Stations 6, 11 and 12 were more or less consistent throughout the year. This distribution indicates that the moderate salinity habitats (Table 1) were nearer the optimum conditions of *Palaemonetes pugio* than were either the lower or high salinity habitats. Therefore, based on all specimens collected, the abundance of *Palaemonetes pugio* is greatest in mesohaline (10–22 ppt) waters and least in fresh water (<1.0 ppt).

The distribution of grass shrimp with regard to salinity is in close agreement with findings of Gunter (1950, p 22). The greatest abundance was in water of 10.0–14.9 ppt salinity, but a large number of shrimp were caught at stations with higher salinity (1, 2, 3, 12 and 13). These data are in good agreement with those of Lofts (1956) which show that *Palaemonetes varians* can tolerate wide salinity ranges.

Effects of Salinity and Temperature on the Abundance of Sexes. Effects of temperature indicate that males and ovigerous females are more abundant when the water is ≥30.0 C and non-ovigerous females are more abundant when the water is 14.0–21.9 C (Fig. 2), suggesting that the reproduction potential is probably correlated with temperature. This correlation is to be expected, since, in a discussion presented later, it is shown that a temperature range of 14.0–21.9 C is too low

Table 2

Total *Palaemonetes* collected from March, 1963 to February, 1964

	March	April	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	
Station	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Total
1	135	506	708	153	112	81	72	273	. .	24	147	2211
2	313	301	228	55	75	18	26	35	. .	5	5	1061
3	1	205	181	242	27	61	179	45	. .	5	144	1090
4	78	85	229	93	11	31	196	72	. .	23	. .	818
5	65	221	847	185	434	367	105	37	. .	1	5	2267
6	182	60	16	1183	960	495	625	535	. .	13	31	4100
7	37	187	80	586	310	377	438	318	69	349	545	3296
8	. .	. .	13	9	1	59	24	. .	11	14	. .	131
9	145	41	169	94	334	195	939	236	. .	. .	37	2190
10	85	72	51	681	329	145	750	53	. .	5	33	2204
11	192	271	112	383	208	31	859	337	166	391	219	3169
12	168	216	576	1512	510	579	692	310	. .	. .	259	4822
13	231	579	581	1094	232	58	64	286	13	. .	123	3261
Total	1632	2744	3791	6270	3543	2497	4969	2537	259	830	1548	30620

for most *P. pugio* to reproduce. The numbers of males and ovigerous females increase significantly with increases in salinity, whereas, the non-ovigerous females decreases (Fig. 3).

The appearance of ovigerous females in March (when the temperature rose to 18–20 C) and their disappearance in October (when the temperature dropped below 18–20 C) was slightly different but included the months of reproduction shown by Gunter (1950), Lofts (1956), Gurney (1923) and Broad and Born (1963). However, the longevity of the reproduction season agrees closely with that of Meehean (1956), who showed that ovigerous females were present from March to late September. Also, the presence of significant numbers of males in relation to females throughout the year differs from the data of Broad and Born (1963) who indicate that the males of *P. kadiakensis* decreased markedly in abundance in June.

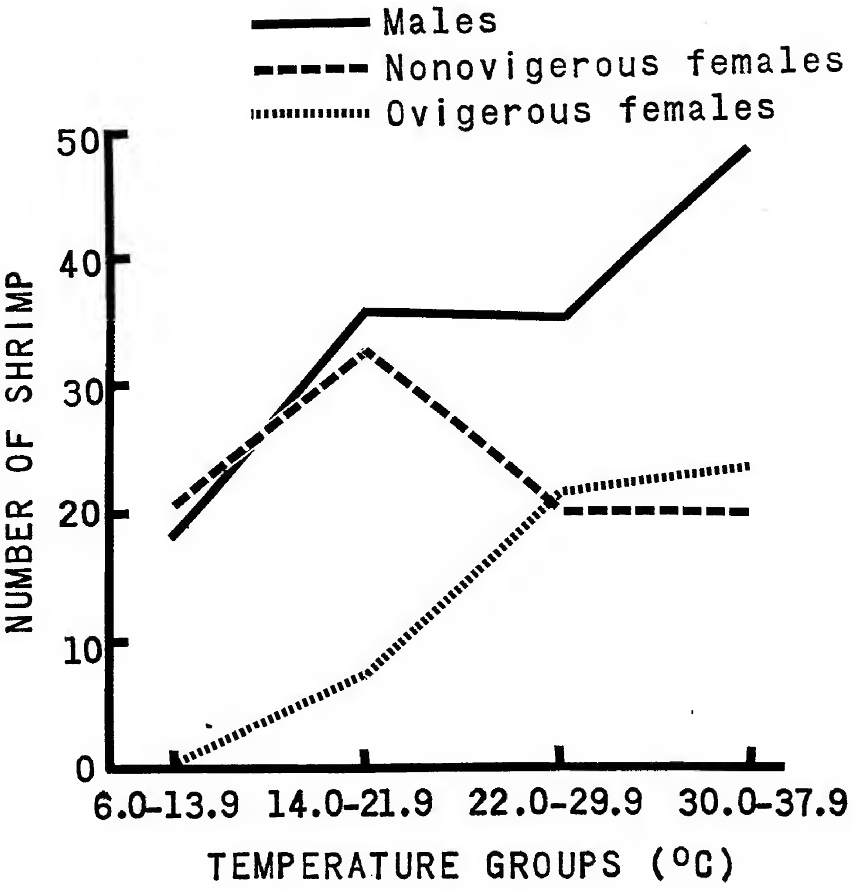

FIG. 2. Average number of males, non-ovigerous females and ovigerous females of *P. pugio* collected at the indicated temperatures.

RELATIONSHIPS OF ENVIRONMENTAL AND ECOLOGICAL CONDITIONS TO THE LENGTH-FREQUENCY DISTRIBUTION OF *P. pugio*

The population growth of two age groups was traced from the estimates of length-frequency data (Fig. 4).

The similarity of characteristics shown by the length-frequency distributions (Fig. 4) suggestes that the samples present a reasonably accurate picture of the annual growth and life history of *P. pugio*. These characteristics are: 1) orderly progression of modal groups, 2) regression of size in late summer and fall and 3) similarity of approximate size shown by shrimp sampled in the early spring.

The bimodal curve of average total length of *P. pugio* indicates that growth to adult size is rapid (2–3 months) in warmer months (June–September), while in the colder months it takes 4–6 months (Fig. 4). Peaks of modal progression at about 27–30 mm suggest that longevity of each shrimp is confined to a single year. Data in Figure 4 suggest that the first age group appears in spring and early summer. Their development can be traced to sexual maturity in late summer and until they reach maximum size at about the time the first eggs are laid in the spring. The second group may be traced from postlarvae in the fall until early the next spring and maturity early in the following summer. The first group, or larger individuals, die off after spawning, leaving those older shrimp which re-

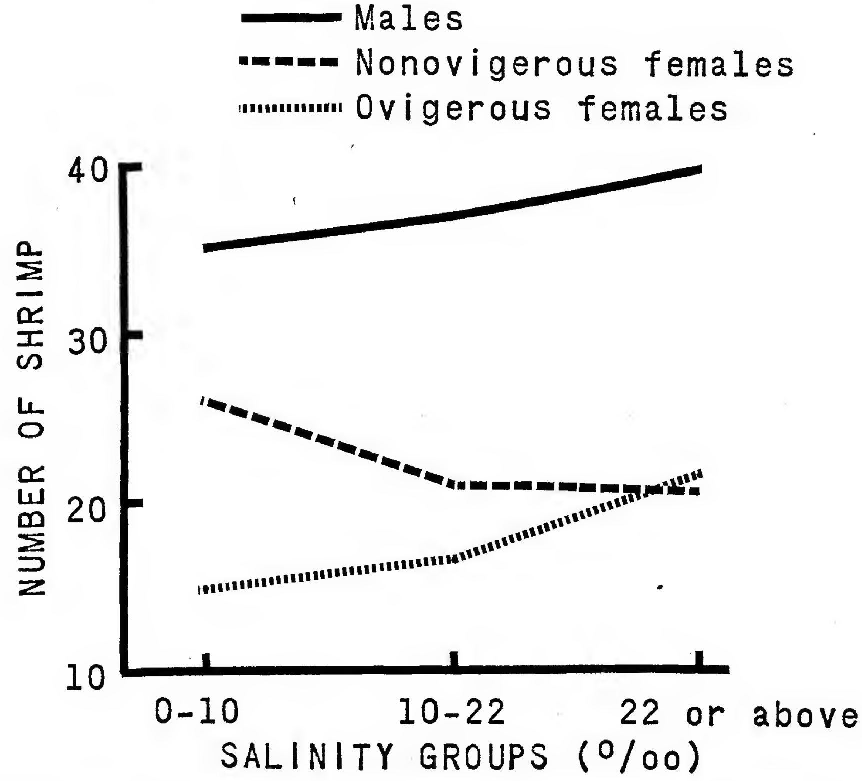

Fig. 3. Average number of males, non-ovigerous females and ovigerous females of *P. pugio* collected from the indicated salinities at each station.

produce late and the smaller second group to spawn in the early summer and again in the fall.

There is an apparent size difference between males, non-ovigerous females and ovigerous females. This difference is accentuated in the longer, and presumably older, individuals. Average length of all males was 23.5 mm, of non-ovigerous females, 26.2 mm and of ovigerous females, 30.0 mm. The largest female captured was 42.0 mm long whereas the largest male collected was 31.0 mm long.

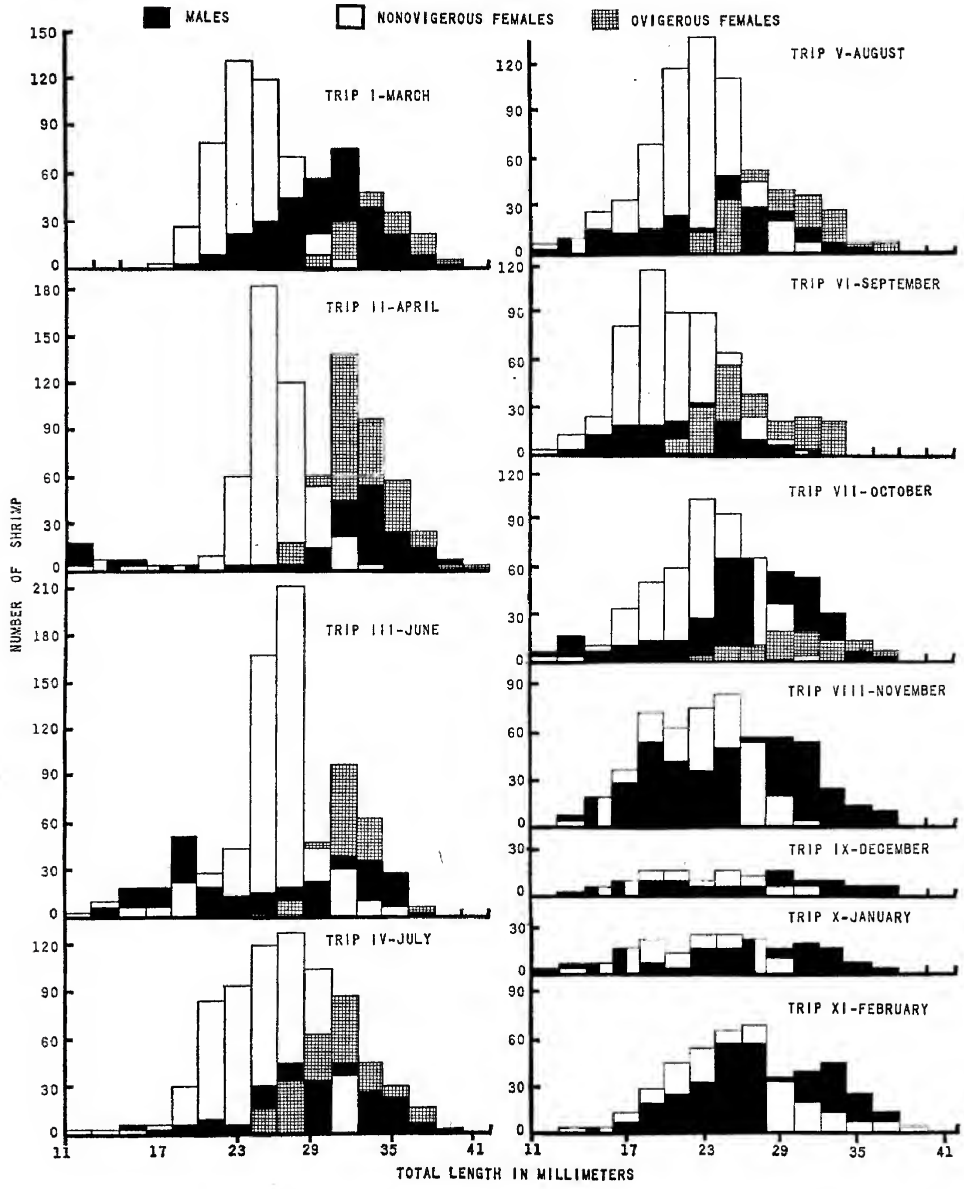

Fig. 4. Length-frequency of *P. pugio* from the Galveston Bay system.

Plots of the effects of field temperatures and salinities on the size distribution of *P. pugio* show that length is proportional to temperature. Largest grass shrimp were found when the water temperature was 30 C or above and the salinity 10 to 22 ppt and the smallest at low temperatures (6.0 to 13.9 C) and salinity 0 to 10 ppt (Fig. 5).

No statistical significance was noted between salinities, although there were more large (30–34 mm) ovigerous females in high salinity water than were present in lower salinities. This might be due to a migration of some of the non-ovigerous females to waters of lower salinities.

EFFECTS OF TEMPERATURE AND SALINITY ON LENGTH INCREASE OF THE SEXES OF *P. pugio*

Although the original design of the study was not intended to reveal the size differences of the individual sexes of *P. pugio*, data showed that time, temperature and salinity have greater effect on the length-frequency of the different sexes of grass shrimp than habitat. Increments of 2 mm in lengths of each sex were plotted to indicate the effect of time (Fig. 4). A weighted analysis of variance and Duncan's tests were conducted on the sum-total-mean-length measurements of each sex for each trip, station, temperature and salinity group.

Effects of temperature changes on length increases in both sexes indicate that there was a significant increase in length between the coldest and warmest months in the field in both sexes of *P. pugio* and that there was no appreciable increase in

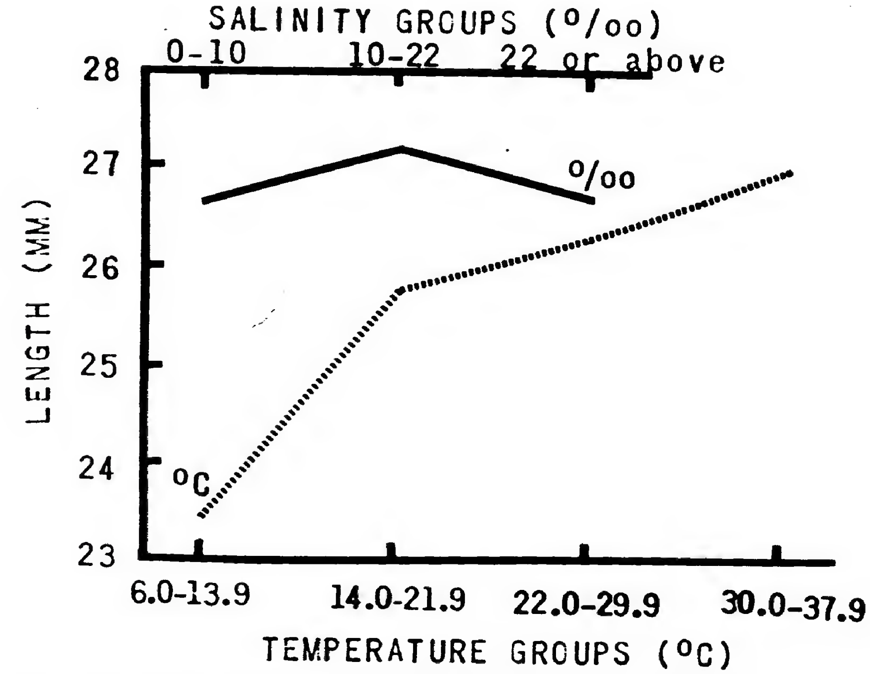

Fig. 5. Average length of all *P. pugio* at the indicated temperatures (dotted line) and salinities (solid line).

length between the moderate and warmest field temperature groups in both sexes of *P. pugio*. Ovigerous females showed the greatest increase in length between the different temperature groups.

Salinity has no statistically significant effect on the mean total length of males, non-ovigerous females and ovigerous females of *P. pugio*. However, length of males at low salinity was found to be significantly different from those at moderate and high salinities, and the lengths of the ovigerous females at <10 ppt were significantly larger than those at >22 ppt. There was no significant length difference in the non-ovigerous females. Fewer, but larger, females (36–42 mm) were found mainly in 0–10 ppt salinity; more abundant, but slightly smaller, specimens (28–34 mm) were found predominantly in higher salinities. Also, more and larger males were found predominantly in more saline waters.

REPRODUCTION CHARACTERISTICS

Sex Ratio. In the 121 samples collected, totaling 9043 grass shrimp, there were 4417 females (both ovigerous and non-ovigerous) and 4626 males, a ratio of 51.2% males to 48.8% females. The % occurrence of each sex fluctuates, however, with spawning and with temperature (Fig. 6).

During summer, males were dominant and there was fluctuation in the ratio of

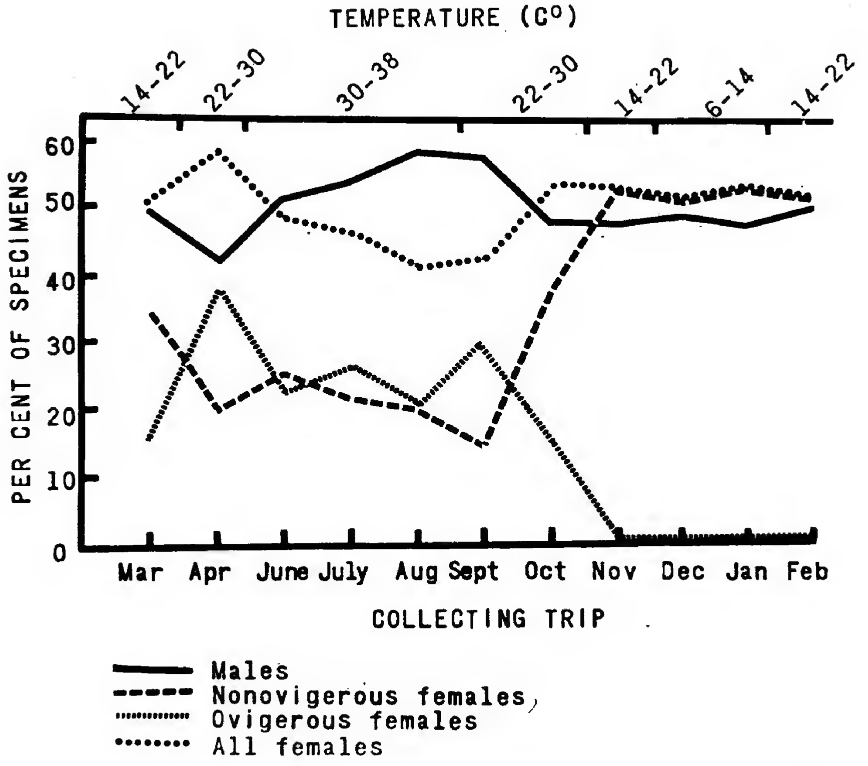

Fig. 6. Percent-frequency of *P. pugio* at the indicated temperatures and collecting trip.

the sexes. During the winter, the proportion of males to females was constant, but the proportion of the sexes was reversed.

The ratio of non-ovigerous to ovigerous females tended to be reversed when the temperature increased to above 22 C.

Salinity had little effect on the ratios of males to females. However, when the two types of females were considered separately, the ratio of non-ovigerous females to ovigerous females was about 4 to 1 at low salinity, and about 2.5 to 2.0 at high salinities.

Spawning. Ovigerous females were collected from March to October, 1963 in waters having a temperature range of 17.4 to 37.5 C and a salinity range of 2.3 to 33.9 ppt. The smallest berried female was 18.5 mm long while the largest was 42.0 mm long.

Seasonal temperatures may contribute to two obvious pulses of spawning, one in the early summer and one in the early fall. A theoretical presentation of these spawning periods is shown in Table 3.

The effect of temperature on reproduction of *Palaemonetes* supports Orton's (1920) conclusions that most animals under normal conditions begin to breed either at a definite temperature or a definite temperature change. A minimum temperature of about 18–20 C is required to initiate reproduction, but the greatest amount of spawning occurs when the water temperature is 22 C. This "semi-annual" spawning cycle agrees closely wih the spawning cycle of *P. paludosa* in Louisiana (Meehean 1956), but differs from the spawning cycles of *P. kadiakensis* and *P. exilipes* in Ohio (Broad and Born 1963; Gebhart 1936) and *P. varians* in Britain (Lofts 1956; Gurney 1923) where spawning occurs once a year in early summer. Differences in the length of the spawning cycles are due probably to the seasonal temperature differences found at different latitudes.

Effects of salinity on spawning is probably included in a temperature-salinity interaction effect on spawning. When grass shrimp spawned in early summer, temperature was high (30 C) and salinity was moderate (15–22 ppt), whereas, in the fall, temperature was moderate (15–25 C) and salinity high (>22 ppt).

Reproductive Potential. Only larger females (32–38 mm) produced egg masses during March to June. These large females were replaced gradually as egg bearers by progressively smaller individuals. The smaller berried females (20–31 mm) appeared during August and September. This trend reverted until spawning terminated in late October. This agrees closely with the findings of Meehean (1956).

The number of eggs carried by a female increases with body length (Fig. 7).

TABLE 3

Spawning cycle of *P. pugio* in the Galveston Bay system

March–July	August–October	October–March	March–July
A_0 and/or $A_1 + A_2$	A_1', but with some A_2	$A_1' + A_2'$ (approx. equal)	A_0' and/or $A_1' + A_2'$

A_0 = parent generation.
A_1 = generation spawned in spring of previous year.
A_2 = generation spawned in fall of previous year.
A_1' = generation spawned in spring of present year.
A_2' = generation spawned in fall of present year.
A_0' = parent generation of the following or future year.

This relationship is non-linear, and a semi-log transformation was used, taking the logarithm of the number of eggs, to express the function as a linear regression of number of eggs to body length. The relationship is represented by the following equation:

$$\log Y = 1.19 + 0.0408X,$$

where Y is the number of eggs and X is the total body length in millimeters. This agrees with Jensen's (1958) findings on the relation between body size and number of eggs in marine Malacostraca, but differs greatly from Meehean's (1956) egg counts for *P. paludosa.* A possible explanation for this large difference in egg numbers may be a physiological process similar to that described by Solland (1923) to explain why eggs from estuarine *Palaemonetes* were smaller than those of fresh water grass shrimp.

The average number of eggs that each female carried during different times of the year indicates that the production was highest during June and October when larger females were bearing eggs (Fig. 8). Numbers of eggs per berried female

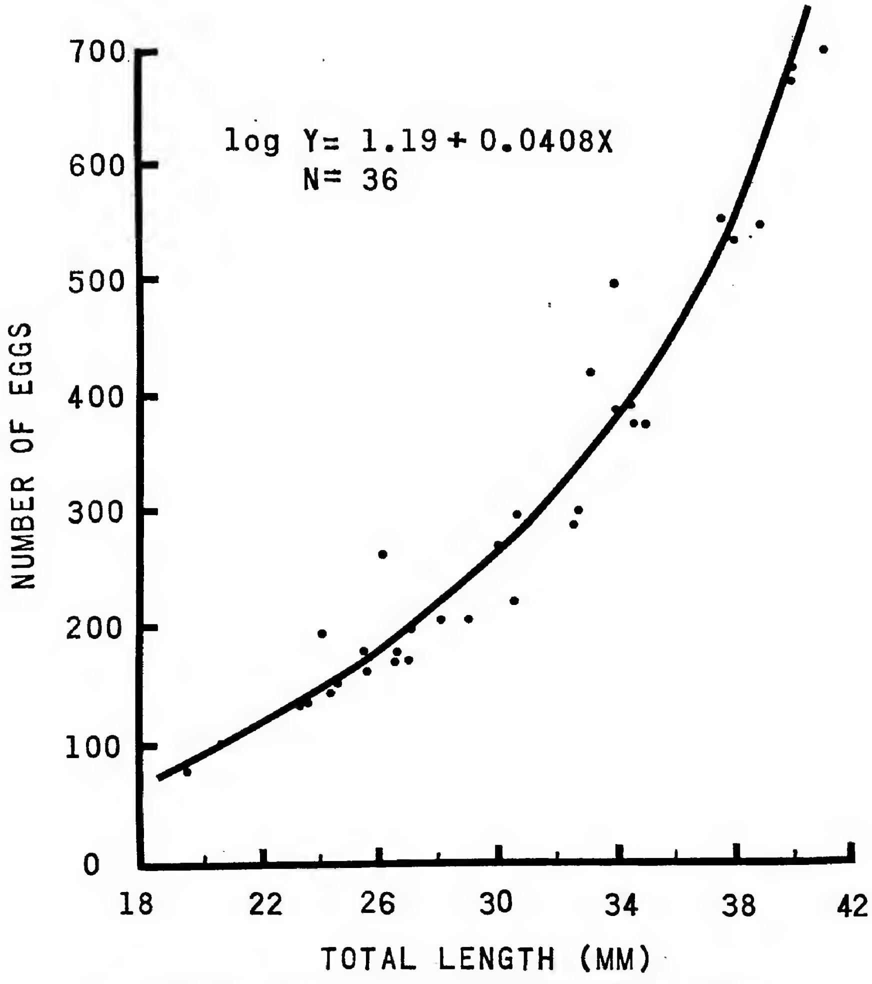

Fig. 7. Number of eggs in egg mass as related to total length of female.

were lower in August-September when the females were smaller. Lack of quantitaitve data on the size of the population makes it impossible to indicate the magnitude of the spawning peaks. It would appear from Figure 8, however, that about 1.5 times as many small ovigerous females in August and September and 1.25 ovigerous females in October would be necessary to equal the egg production of the large ovigerous females in the spring and early summer.

Peak egg production coincides with months when highest percentages of gravid females appeared; however, the percent of ovigerous females is not a reliable criterion for judging the egg production of the population. For example, from Figure 6 it can be estimated that 23.0% of females bore eggs in June and 28.0% bore eggs in September, whereas the calculated mean number of eggs per ovigerous female (Fig. 8) was 372 in June and 198 in September. Therefore, the larger egg masses carried in June present a higher index of population production than the smaller egg masses spawned in September, even though the percentage of females bearing eggs in September was appreciably higher than in June.

LABORATORY EXPERIMENT

The second phase of this study was designed to determine the effect of temperature and salinity on growth and survival of *P. pugio* in the laboratory. This study was conducted concurrently with field studies.

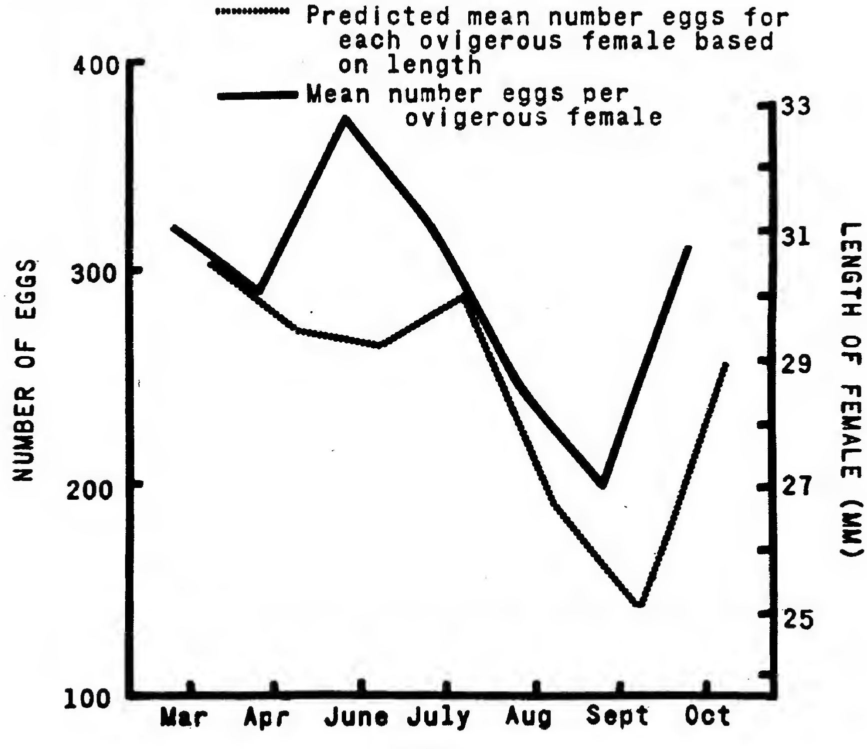

FIG. 8. Mean and predicted number of eggs per ovigerous female based on length and actual number of eggs.

TECHNIQUES

Procedures were similar to those used by Zein-Eldin (1963) for rearing penaeid postlarvae. Sixteen groups of young were reared from eggs and held in aquaria for 30 days at all combinations of four salinity levels (4–10, 10–16, 16–22, and 22–28 ppt) and four temperatures (11, 18, 25, and 32 C) under controlled laboratory conditions, to approximate field conditions. Each group of shrimp was acclimated to the desired salinity and temperature over a period of four to five days.

Each aquarium was fitted with "Eureka" plastic bottom filters, which were covered with ground oyster shell topped with layers of medium and fine sand. This combination served both as a filter bed and as a substrate for the shrimp. Both shell and sand were washed and heat-sterilized before use.

During the experiment, the temperature never varied more than ± 0.5 C in each test, and the salinity never exceeded the experimental parameters specified. Distilled water was added to counteract salinity increases caused by evaporation.

Zoaeal and postlarval *P. pugio* were fed nauplii brine shrimp during the experiment because they are preferred as food (Broad 1957b) and live food helps prevent fouling. The volume and number of feedings per day were increased during the experiment in order to maintain an excess of food in the tanks at all itmes.

Length and weight measurements were obtained from samples of 10 grass shrimp randomly drawn from the source "population" at each salinity level before temperature acclimation. After execess water was removed, the length of each specimen was measured with the aid of a micrometer to the nearest 0.5 mm, and its weight determined on the Mettler H-15 analytical balance to the nearest 0.1 mg. Subsequent samples taken randomly from each experimental tank were similarly treated at six-day intervals. After 30 days, the surviving shrimp in each tank were counted.

SURVIVAL STUDY

A survival study was included in the growth experiment to obtain information on the effects of environmental factors on the survival of *P. pugio* postlarvae and juveniles. Because some of the shrimp were removed and not replaced during the growth experiment, effects of salinity and temperature differences were estimated on the total percent surviving, assuming that those withdrawn would have survived.

Both temperature and salinity affect the survival of *P. pugio*. Grass shrimp can survive over prolonged periods (30 days) at low-moderate temperatures (18–25 C) with no appreciable amount of mortality (Table 4). However, shrimp held for prolonged periods (30 days) at 11 C have a high rate of mortality. *P. pugio* survives best in oligohaline to mesohaline waters, but many (70%) can survive in low metahaline water (Table 4). This is in close agreement with the findings of Loft (1956) which indicate that *P. varians* can survive over a wide range of salinity.

Interactions of temperature and salinity on total survival indicate that water with temperatures of 18 C or above and salinities of 4–16 ppt are best suited for survival. Waters with lower (11 C or below) temperatures and higher salinities (22–28 ppt) result in lowest survival (Table 4).

GROWTH STUDY

Both temperature and salinity affect metabolism and hence growth and distribution of all cold-blooded animals, such as the grass shrimp (Pearse 1939; Allee *et al.* 1949; Verwey 1957; Gunter 1957; Moore 1958; and Waterman 1960). However, few data are available to show the importance of temperature and salinity interactions.

Panikkar (1941), Lockwood (1962) and Dobkin and Manning (1964) indicate that *Palaemonetes* has a highly developed osmoregulatory ability and is osmotically independent. *Palaemonetes* in general have the ability to regulate their permeability to water and increase the assimilation of ions in the blood so as to remain hyperosmotic to the medium when they are in dilute solutions and hypo-osmotic when in a concentrated medium. According to Zein-Eldin (1963), postlarval penaeid shrimp can both survive and grow over a wide range of salinities. Also, Williams (1960) found that temperature had a far greater effect upon survival of penaeid shrimp than did salinity.

P. pugio can grow over a wide range of salinities and temperatures .However, increases in both length and weight were greatest in waters with temperatures between 25 and 32 C, and salinities between 16 and 22 ppt; the least amount of growth occurred at low temperatures (15 C or lower) and salinities between 22 and 28 ppt (Figs. 9 and 10). Growth was negligible at all salinity interactions involving 11 C temperature. Greatest growth occurred from 18 to 30 days after the grass shrimp became postlarvae and weight increased more rapidly than length. This type of growth relationship was also shown for the penaeid postlarvae reared in the laboratory by Zein-Eldin (1963).

The possibility and, indeed, the reality of interactions of temperature and salinity on growth in time must be considered. Due to some of the types of comparisons made and because there was an interaction between the temperature and salinities tested, a weighted error term was used in computing the confidence range values of some of the Duncan Multiple Range Tests.

An analysis of variance and Duncan Multiple Range Tests were conducted on both length and weight measurements to indicate the influence on growth by: (1) time at all combinations of temperatures and salinity, (2) changes in salinity at each of the six elapsed measuring times, (3) changes in measuring time at each of the four salinities, (4) changes in temperature at each of the elapsed measuring

TABLE 4

Number and per cent (in parentheses) of *P. pugio* surviving various temperature-salinity conditions. (60 shrimp = 100% survival)

	Salinity range (ppt)				
	4–10	10–16	16–22	22–28	Total
32C	54(90)	51(85)	42(70)	39(65)	186(78)
25C	52(87)	55(92)	40(67)	41(68)	188(78)
18C	48(80)	48(80)	48(80)	45(75)	189(79)
11C	35(58)	37(62)	39(65)	31(52)	142(52)
Total	189(79)	191(80)	169(70)	156(65)	

times and (5) changes in measuring time at each of the four temperatures (Table 5 and 6).

The analysis of variance indicates that all factors and their interactions were significant for both length and weight. However, after Duncan tests had been performed on the various interactions mentioned above, it was found that many of these significant values did not exist.

A brief discussion is necessary to indicate just where some of the not so obvious statistical significant differences occurred in Figures 9 and 10.

There was an appreciable increase in both length and weight over time (Figs. 9 and 10). However, only the length and weight obtained in 24 and 30 days was significantly greater than that attained at all other measuring days.

There were no statistically significant increases in lengths or weights as affected by changes in salinity at each of the six elapsed measuring times. However, when a "t" test was performed on the weight increase at the 30-day measuring time (Fig. 9) it was found that all increases at <22 ppt were highly significant with that at >22 ppt salinity.

Some of the length increases after 18 days were significantly greater than those

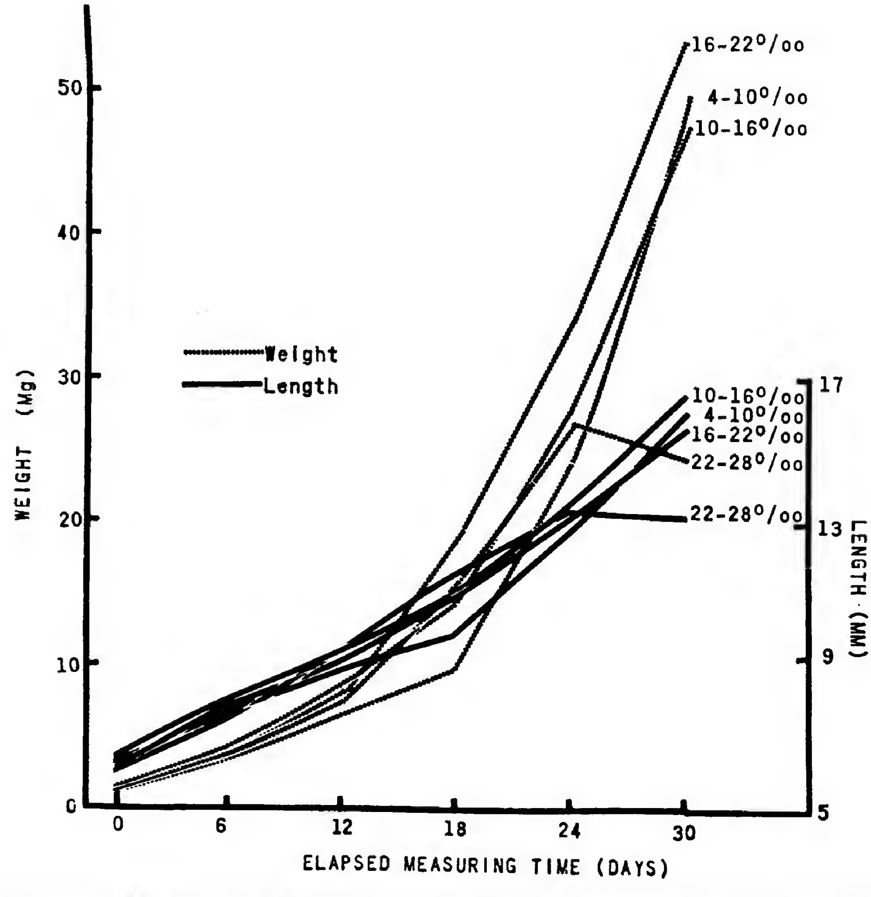

Fig. 9. Average length and weight of *P. pugio* at the indicated experimental salinities and times.

from 0–18 days in all salinities (Fig. 9). Significant increases in weight occurred in all salinities except 22–28 ppt after 18 days. At 22–28 ppt, the weight increase at 30 days was significant only with that obtained from 0–12 days.

There was no appreciable increase in length between the different temperatures at the first three elapsed measuring times (Fig. 10). However, on the 18th, 24th, and 30th days, the length increases at 32 C were significantly greater than increases at 11 and 18 C.

There was no statistically significant increase in weight for the different temperatures on any elapsed day before the 24th day. However, on the 24th day the weight at 32 C was significantly greater than at 18 C and 11 C, and on the 30th day the weights at 32 C were greater than at all the other temperatures.

To show the effects of temperature and salinity interactions on growth (Fig. 11) a weighted analysis of variance (Table 7 and 8) using a weighted error term

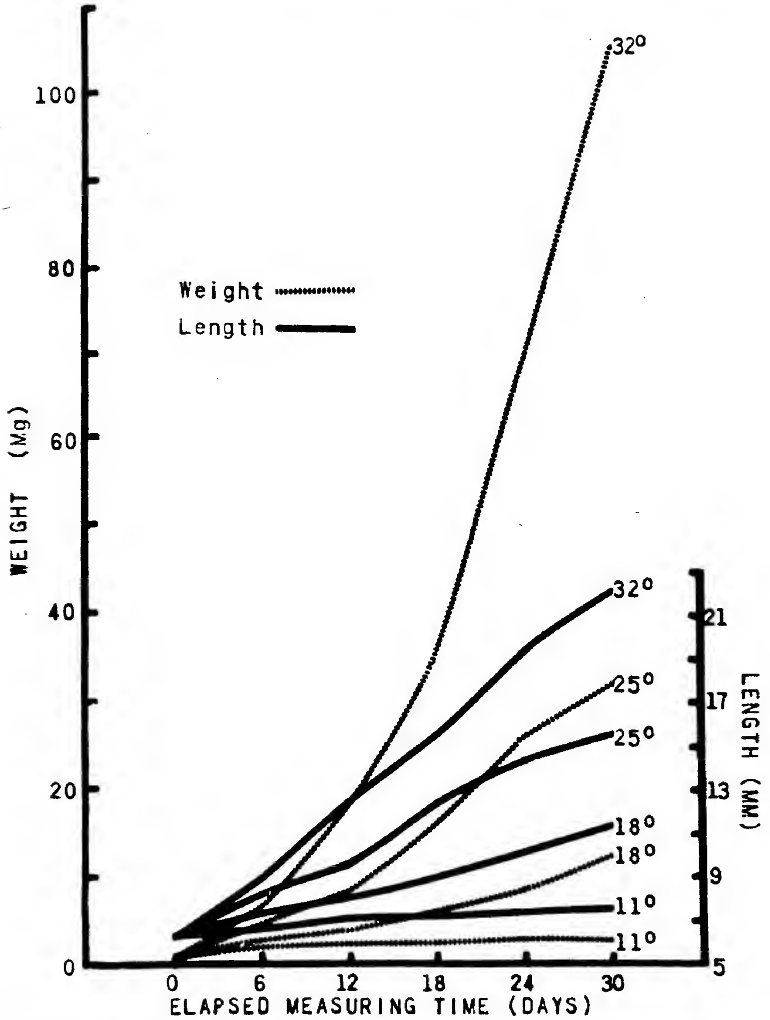

FIG. 10. Average length and weight of *P. pugio* at indicated experimental temperatures and times.

Table 5

Analysis of variance of the length of *P. pugio* under laboratory conditions

Source of variance	Degrees freedom	Sums of squares	Mean square	F test
Total	843	18071.17	21.44	....
Temperature	3	4206.98	1735.66	924.48[a]
Salinity	3	98.12	32.71	17.42[a]
Time	5	5459.71	1091.94	581.61[a]
Temp. × Salinity	9	82.40	9.16	4.88[a]
Temp. × Time	15	2894.06	192.94	102.77[a]
Salinity × Time	15	109.82	7.32	3.90[a]
Temp. × Time × Sal.	45	175.08	3.89	2.07[a]
Individuals (Error)	748	1404.33	1.88	...

[a] Significant at 95% probability.

Table 6

Analysis of variance of the weight of *P. pugio* under laboratory conditions

Source of variance	Degrees freedom	Sums of squares	Mean square	F test
Total	843	0.70602	0.00084	.....
Temperature	3	0.16476	0.05492	279.96[a]
Salinity	3	0.00660	0.00220	11.22[a]
Time	5	0.10535	0.02107	107.41[a]
Temp. × Salinity	9	0.00835	0.00093	4.73[a]
Temp. × Time	15	0.16503	0.01100	56.09[a]
Salinity × Time	15	0.01028	0.00069	3.49[a]
Temp. × Time × Sal.	45	0.01915	0.00043	2.17[a]
Individuals (Error)	748	0.14673	0.00020	.....

[a] Significant at 95% probability.

Table 7

Weighted analysis of variance of length increases under laboratory conditions

Source of variance	Degrees freedom	Sums of squares	Mean square	F test
Temperature	3	5206.98	1735.66	189.57[a]
Salinity	3	98.12	32.71	2.68
Temp. × Salinity (Error)	9	109.82	12.20	...

[a] Significant at 95% probability.

Table 8

Weighted analysis of variance of weight increases under laboratory conditions

Source of variance	Degrees freedom	Sums of squares	Mean square	F test
Temperature	3	0.16476	0.05492	5.90[a]
Salinity	3	0.00660	0.00220	2.37
Temp. × Salinity (Error)	9	0.00835	0.00093	...

[a] Significant at 95% probability.

and Duncan's Multiple Range Tests were conducted on the means of both length and weight measurements. Each temperature-salinity combination was tested to indicate the significant growth as affected by: (1) salinity and temperature, (2) changes in the salinity at each of the four temperatures and (3) by changes in the temperature at each of the four salinities.

None of the increases in length and weights were affected by salinity to the degree of being statistically significant. However, the increases in length between each of the temperatures tested were statistically significant. The increases in weight at 25 C and 32 C were significantly greater than those at 11 C and 18 C.

An appreciable amount of length and weight increase occurred between the different temperature ranges at each salinity (Fig. 11). To better explain the

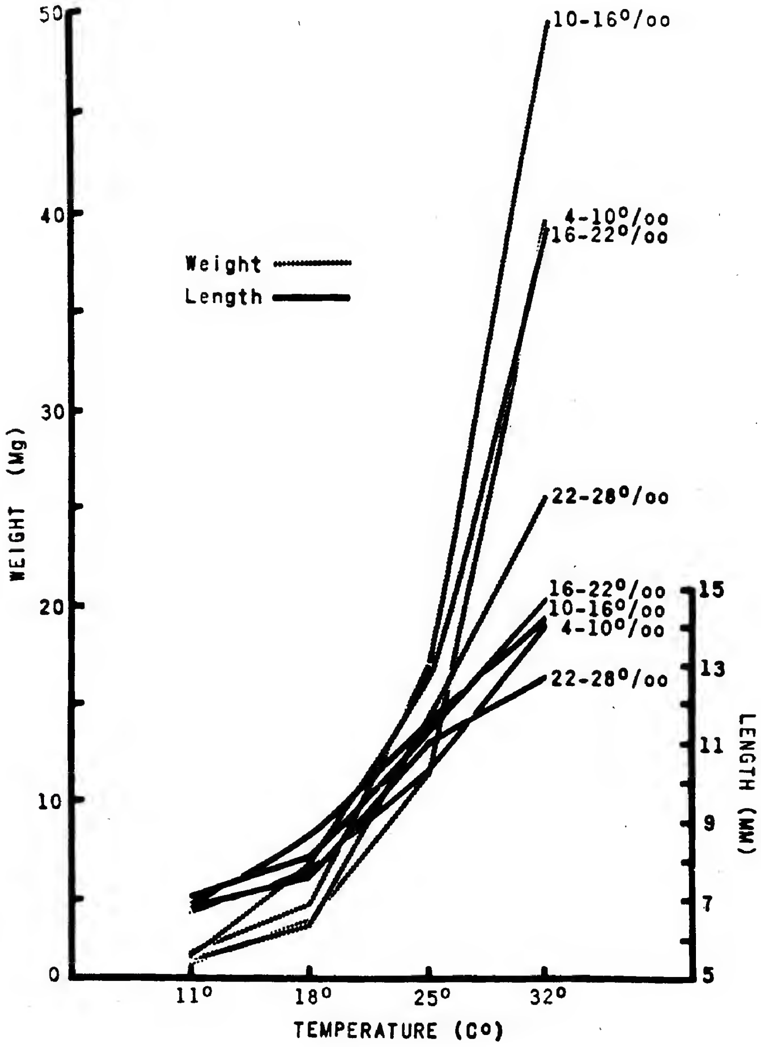

FIG. 11. Average length and weight of *P. pugio* at experimental salinities given for four temperature groups.

data, "t" tests were performed between each temperature at the particular salinity tested. At all salinities except 22–28 ppt, the weight increase at each temperature was significantly greater. At 22–28 ppt salinity, the weight at 32 C was not significant with that at 25 C, but was with increases at 11 C and 18 C. A significant increase in length is noted between each of the temperatures at each of the salinity ranges.

As indicated in the analysis of variance (Tables 5 and 6), there was significant growth in length and weight influenced by salinity, temperature, and the interaction of these two factors. A weighted analysis of variance (Tables 7 and 8) indicated that there are no statistically significant increases in the weights or lengths as affected by changes in the salinity at each of the four temperatures. The F value for length, however, is significant at approximately the 10% level (Table 7); and length increase is found to be statistically significant only for the temperature 32 C where lengths at salinities of 4–22 ppt are different from those at a salinity of 22–28 ppt. At all other temperatures, there was no significant difference between the lengths.

SUMMARY AND CONCLUSIONS

A study was made in two phases of the seasonal growth rate, size difference, reproductive potential, seasonal occurrence and distribution as affected by temperature and salinity of *Palaemonetes pugio* in the Galveston Bay estuarine system.

A field survey of the grass shrimp population in the shore zone was made from March, 1963, to February, 1964.

Grass shrimp were taken in greatest numbers in July and October, although some shrimp were caught on each collecting trip.

The sexes of collected *P. pugio* were found to be related to temperature. Males and ovigerous females were more abundant when the water temperature was ≥30.0 C and non-ovigerous females were more abundant when the water was 14.0–21.9 C.

The abundance of grass shrimp is greatest in mesohaline (10–22 ppt) waters, and least in fresh water (<1.0 ppt). As the salinity increased, males and ovigerous females increased in number and non-ovigerous females decreased in number.

Growth to maturity (20–24 mm) takes two to three months during June–September and four to six months during the remainder of the year. Sexually mature specimens were obtained in 30 days in the laboratory. Thus, there is little doubt that grass shrimp spawned in the spring and early summer can reach sexual maturity by the fall of the same year, and that such individuals may spawn at that time.

Longevity of grass shrimp is about one year.

In regard to growth (length and weight increase), temperature and salinity and their interactions had similar effects on *P. pugio*. In both the laboratory and the field there was no appreciable increase in length that could be attributed to salinity alone. In both laboratory and field, the greatest increase in length occurred in waters where the temperature was ≥ 30 C and the least in waters of 11 to 14 C. Also, the data from the field and laboratory are similar in showing the effect of changes in temperature at different salinity levels. Length increase in the laboratory was greater at 32, 25 and 18 C than at 11 C at each of the salinity ranges, and in the field the lengths at 30–37, 22–30 and 14–22 C were greater than those at 6–14 C. The effects of change in salinity at different temperatures in both the laboratory and field indicate few significant increases in length.

There is a size difference between males (mean length—23.5 mm), non-ovigerous females (26.2 mm), and ovigerous females (30.0 mm) which is accentuated in the longer and older individuals.

Habitat has little influence on the average lengths of *P. pugio*, except in fresh water where the shrimp are much smaller.

P. pugio can survive over a wide range of salinities and temperatures. In the laboratory, waters with temperatures of 18 C and salinities of 4–16 ppt were best suited for survival, and waters with temperatures of 11 C or lower and high salinities (22–28 ppt) showed the lowest survival. However, survival at high temperatures and high salinities was not as high (65%) as that recorded (90%) at high temperatures and lower salinities. This phenomenon was illustrated in the field when the abundance of *Palaemoonetes* decreased at the more saline stations and increased at stations of normally low salinities when the salinity began to increase in July; the numbers of shrimp at stations in the mesohaline salinity range (10–22 ppt) remained constant, indicating that these habitats offered conditions nearer the optimum for *Palaemonetes* than did either the lower or higher saline habitats. In addition, numbers of captured grass shrimp were lower when the water temperature dropped to 15 C or lower.

Length is correlated to temperature, with the largest grass shrimp being found when the temperature in the field was 30 C or above and least at 14 C or below. However, the largest females (both ovigerous and non-ovigerous) were found when the water temperature was 14.0–21.9 C.

Except for those shrimp in fresh water, which were smaller, salinity had little effect on the length or weight of *P. pugio*.

The sex ratio of *P. pugio* was 51.2% males and 48.8% females, or approximately 1:1. Males were the dominant sex in summer, while in the winter the females were more abundant. Also, as the temperature increased above 18–20 C, ovigerous females became the dominant female type.

Salinity had little effect on the ratio of males to females; however, when the 2 types of females are considered separately, the ratio at low salinity for non-ovigerous to ovigerous females was 4:1 and at high salinities approximately 2.5:2.

Ovigerous females were collected from March to October in a temperature range of 17.4 to 37.5 C and a salinity range of 2.3 to 33.9 ppt. The smallest ovigerous female was 18.5 mm in length while the largest was 42.0 mm.

There were two peak spawning seasons, one in early summer (July) and the other in the early fall (October). Water temperatures of approximately 18–20 C are required for reproduction to occur.

The effects of field temperature-salinity interactions on spawning may possibly be explained by examining the survival data taken in the laboratory. As mentioned before, when the grass shrimp spawned in the early summer, the temperature was high and the salinity ranged from low to moderate, whereas in the fall the temperature was moderate and the salinity high. Survival rate is higher at low salinities and high temperatures or when temperature is moderate and salinity is high. The peaks of spawning, therefore, fall in June and October when the "optimum" conditions for survival are present.

The largest females (28–42 mm) produced egg masses in the spring and early summer, but were gradually replaced by progressively smaller individuals in August and September when the trend reverted toward larger ovigerous females. The number of eggs carried by ovigerous females increased in a geometric progression related to her length. The number of eggs contained in an egg mass is a linear function of the female's length and is represented by the formula log $Y = 1.19 + 0.0408X$.

ACKNOWLEDGMENTS

I am particularly grateful to Dr. Richard J. Baldauf, Department of Wildlife Science, who directed the original work and offered many constructive suggestions. Special thanks are due to the staff members of the U. S. Bureau of Commercial Fisheries Laboratory, Galveston, Texas for their technical assistance and helpful suggestions with regard to the manuscript. Finally, I would like to thank my wife, JoAnn, for financial support, never-ending encouragement, aid in recording data, typing and proof-reading of the original thesis and this manuscript.

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A REVIEW OF THE RECENT MARINE PODOCOPID AND PLATYCOPID OSTRACODS OF THE GULF OF MEXICO

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ABSTRACT

Taxonomic, ecological and other studies on Gulf of Mexico ostracods are discussed and summarized. The salinity, temperature, depth ranges, sediment type and environment for most named Recent species reported from the Gulf are given.

INTRODUCTION

The Recent marine ostracods of the Gulf of Mexico have been studied more intensively than in any other geographic area in the world. The Gulf of Mexico is defined as the area within 81.0° and 98.0° W. Longitude and 21.5° to 25.2° and 30.5° N. Latitude. A total of 31 published and unpublished works dealing, in most cases, directly with taxonomy and/or ecology of the marine podocopid and platycopid ostracods of the Gulf of Mexico have been selected for review. Some of the works are unpublished, *i.e.*, in the form of theses or dissertations (Engel 1956; Hulings 1958a; Waller 1961; Morales 1965). These reports contain largely ecological data with little or no regard to taxonomy. Published papers, some of which occur in rather obscure journals, contain a variety of ecological and taxonomic information. In three cases, information is available only in abstract form (Rothwell 1949; Kornicker and Wise 1960b; Kane 1961).

A total of 91 genera of Recent podocopid and platycopid ostracods have been reported from the Gulf of Mexico. The number of named species that have been reported totals 265 but this total includes, according to the best estimate, 24 species that have been placed under two or more genera. The total of un-named species, *i.e.*, species designated as sp. A, B, or sp. 1, 2, etc., is 146. Five named subspecies or varieties and three un-named subspecies or varieties have been reported. Some genera and species of podocopid ostracods that are not typically marine are included in the above totals.

It seems desirable, therefore, to synthesize taxonomic, ecological and other studies that have been conducted on the Recent marine ostracods of the Gulf of Mexico. The taxonomy is in a state of confusion and a number of problems exist. It is hoped that this review will bring the problems into focus and stimulate investigators to seek solutions. There is a mass of ecological information available, and although much of it is of questionable value, some of the data can possibly be used in the interpretation of paleoenvironments.

GEOGRAPHIC CONSIDERATIONS

Various geographic areas, designated by numbers, within the Gulf of Mexico where ostracods have been studied are shown in Figure 1. A breakdown of the

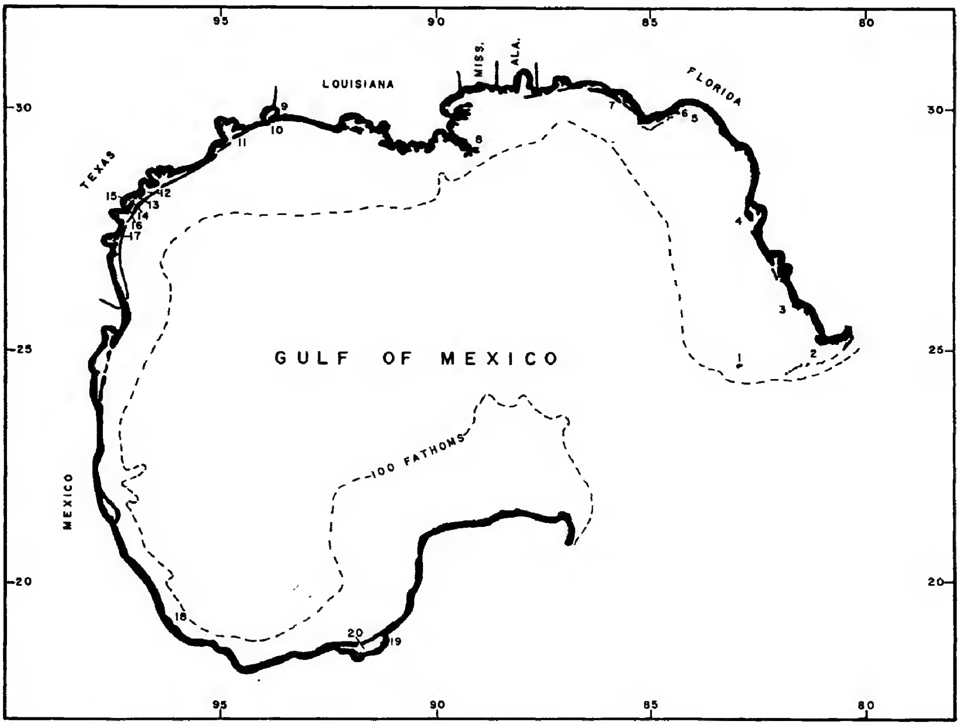

FIG. 1. Geographic areas in the Gulf of Mexico where ostracod studies have been conducted. 1—Tortugas; 2—Florida Bay; 3—Cape Romano; 4—Boca Ciega Bay; 5—Apalachee Bay; 6—Ochlockonee Bay; 7—St. Andrews Bay; 8—East Mississippi Delta; 9—Sabine Lake; 10—Sabine Pass; 11—Bolivar Point; 12—San Antonio Bay; 13—Mesquite Bay; 14—Aransas Bay; 15—Copano Bay; 16—Redfish Bay; 17—Laguna Madre; 18—Veracruz; 19—Carmen; 20—Laguna de Terminos.

literature reveals that the Gulf coastal area of Florida (Tressler 1949; Puri and Hulings 1957; Hulings 1958a;b; Puri 1960; Waller 1961; Benda and Puri 1962; Benson and Coleman 1963; Hulings and Puri 1964) and the Texas Coast (Swain 1955; Engel 1956; Kornicker and Wise 1960b; Kane 1961; Kornicker 1964; Grossman 1965; Keith and Hulings 1965) have been the most studied. Six papers contain information on ostracods of the Mexican Coast (Brady 1870; Sandberg 1964a;b;c; Morales 1965; 1966). A paper by Curtis (1960) deals with the ostracods of the east Mississippi Delta off the coast of Louisiana. Rothwell (1949) also provided data on ostracods off the coast of Louisiana. Tressler (1954) reviewed the knowledge of Gulf of Mexico ostracods and based the review largely on one of his previous papers (1949) and the study by Rothwell (1949).

Most of the studies of ostracods of the Gulf of Mexico have been conducted in marginal marine and/or shallow open marine environments. The studies dealing primarily with the ostracods of marginal marine environments include Kornicker and Wise (1960b), Kornicker (1964), LeRoy (1964), Sandberg (1964b;c), Grossman (1965) and Morales (1965; 1966). The papers by Tressler (1949), Puri and Hulings (1957), Puri (1960), Benson and Coleman (1963) and Keith and Hulings (1965) are concerned primarily with open marine ostracods. Other papers which contain information on both marginal and open marine ostracods include Swain (1955), Engel (1956), Hulings (1958a;b), Curtis (1960), Kane (1961), Waller (1961), Benda and Puri (1962) and Hulings and Puri (1964). All of the open marine studies mentioned above were conducted on the continental shelf and in most cases less than 50 m. Tressler's (1954) paper contains the only information on bathyal and abyssal ostracods of the Gulf. Much of the information in Tressler's paper is taken from the unpublished preliminary work of Rothwell (1949). Tressler also includes the species reported by Brady (1867–87) from the Caribbean. Caribbean species, however, are not considered in the present paper.

Inclusive salinity, temperature and depth ranges, of the sediment type or types and the environments for the majority of the named species reported from the Gulf of Mexico are summarized in Table 1. Also included are the author or authors reporting the species and whether or not illustrations of carapaces and/or appendages are given (asterisk indicates illustrations are provided). The species are given exactly as reported in the literature and no attempt has been made to resolve synonymy of named species. Un-named species have not been included in the table although such information may be of value especially at the generic level. Thirteen genera with no named species have been reported from the Gulf including *Astenocypris* (Swain 1955; Kane 1961), *Brachycythere* (Puri and Hulings 1957), *Buntonia* (Engel 1956; Curtis 1960), "*Favella*" (Engel 1956), *Henryhowella* (Waller 1961), *Hermanites* (Curtis 1960), *Microxestoleberis* (Engel 1956; Curtis 1960), *Monoceratina* (Swain 1955; Puri and Hulings 1957), *Neomonoceratina* (Swain 1955), *Occulocythereis* (Curtis 1960), *Pontocythere* (Puri and Hulings 1957), *Pterygocythere* (Curtis 1960) and *Trachyleberis* (Engel 1956; Curtis 1960).

Authors reporting un-named species include Swain (1955), Engel (1956), Puri and Hulings (1957), Hulings (1958a), Curtis (1960), Puri (1960), Kane

(1961), Waller (1961), Benda and Puri (1962), Hulings and Puri (1964), Kornicker (1964), Keith and Hulings (1965) and Morales (1965).

TAXONOMIC STUDIES

There have been few strictly taxonomic studies on the Recent marginal and open marine podocopid and platycopid ostracods of the Gulf of Mexico. Most of the studies at the species level have been based solely on carapace morphology (Brady 1870; Puri 1958a; 1960; Benson and Coleman 1963; LeRoy 1964; Morales 1965). These workers have described 45 of the 58 new species reported from the Gulf. Tressler (1949), Swain (1955), Sandberg (1964c) and Grossman (1965) based description of new species, subspecies and varieties on carapace and appendage morphology of the remaining 13 new species reported from the Gulf of Mexico. Swain (1955), Benson and Coleman (1963) and Grossman (1965) have described new subspecies and/or varieties.

Five new genera have been described by Puri (1958a; 1958b; 1960) including *Hulingsina*, *Megacythere*, *Neocaudites*, *Protocytheretta* and *Reticulocythere* from the Gulf of Mexico. Benson and Coleman (1963) described *Loxocorniculum* and Sandberg (1964c) revised the genus *Cyprideis*. Puri (1958a;b) revised two subfamilies, Neocytherideidinae and Cytherettinae and erected a new subfamily, Campylocytherinae (1960).

It is obvious from the literature and to the ostracod workers that a number of taxonomic problems at the species level exist within the Gulf of Mexico. Included are the paleontological vs. the zoological approach, *i.e.*, using only carapace morphology as opposed to using both carapace and appendage morphology. Fortunately more modern-day ostracod workers are aware of this problem due, in part, to the Naples Symposium and the subsequent publication of the papers and discussion sessions of the symposium (Puri 1964).

A number of authors have pointed out the relationship of the Recent ostracod fauna of the Gulf of Mexico to Tertiary, mostly Miocene and post-Miocene, faunas (Puri and Hulings 1957; Hulings 1958a; Puri 1958a;b, 1960; Benson and Coleman 1963; Sandberg 1964b) and certainly most workers imply the relationship by using fossil species names. Various authors have reported species in the Recent originally described from Miocene deposits in Florida, North Carolina and Maryland and the Pleistocene of South Florida. Of the 239 named species (the total excludes duplications) from the Gulf of Mexico, approximately 26% are species originally described from Miocene and post-Miocene deposits. There is little doubt that, based on carapace morphology, certain Miocene and post-Miocene species range into the recent. It seems, however, that unless the species in question can be traced through the geologic column to the Recent, then its conspecificity is questionable.

Regarding species described from the Recent, approximately 46% of the Recent species reported from the Gulf of Mexico are species described from geographic areas other than the Gulf. Over one-half of these species were originally described from various European localities. Other areas represented include a variety of localities in the Pacific, South Atlantic and Africa. The conspecificity of a high percentage of the "non-endemic" Gulf species is questionable. Hulings (1966a; b) and others have questioned this concept pointing out that podocopids lack planktonic larval stages, and as a result have no transporting mechanism. It is more logical to relate modern species to fossil species of the same area provided the condition mentioned above can be resolved.

Obviously, other taxonomic problems exist such as the indication from the literature that the fauna of Florida, the Mississippi Delta and the Texas Coast are really different. Modern and classical approaches to taxonomic studies will help resolve these and other taxonomic problems, thereby making ecological studies more significant. The work of Kornicker and Wise (1960a), Kornicker (1961), Sandberg (1964a;b;c), Grossman (1965), Darby (1965), Kesling (1965) and Smith (1965) represents significant advances toward the solution of taxonomic problems.

Hulings (1959; 1967) pointed out the close relationship of the fauna of the southern Atlantic coast of the U.S. and the Gulf of Mexico, especially at the generic level. Benson and Coleman (1963) indicated a close relationship between their southern carbonate shallow-shelf biofacies (Florida Bay area) with the Bahaman-Cuban fauna. Baker and Hulings (1966) found a close relationship between ostracods of the tropical portions of the west coast of Florida and Puerto Rico. The Trachyleberidae were found to be the dominant species around Puerto Rico as they are along the lower west coast of Florida. The two dominant genera in the Puerto Rican fauna were *Bairdia* (17 species) and *Xestoleberis* (19 species). The number of species of these two genera, considerably greater than has been reported from the Gulf, indicates wide diversity. Both genera, however, need to be studied in more detail.

ECOLOGICAL STUDIES

General—Ecological studies of marine ostracods of the Gulf of Mexico are more numerous than purely taxonomic or other types. In most cases attempts have been made to correlate distribution of species with various environmental factors such as salinity, temperature, sediment type, organic content, physical energy, etc. Ostracod assemblages have also been established, especially in marginal marine environments. In many instances differences in the interpretation of distribution vs. environmental factors and environments make it difficult to correlate the results from one geographic area to another.

The summary of selected environmental factors for the named species reported from the Gulf of Mexico given in Table 1 provides basic information for establishing uniform assemblages in the Gulf once the taxonomic problems are resolved. Only these environmental factors most commonly measured were selected. Although other factors are significant, there is not enough information at present to properly evaluate their role.

Various authors have singled out an environmental factor or factors as controlling the distribution of ostracods in the Gulf. Rothwell (1949), according to Tressler (1954), used depth to establish biozones. Swain (1955) and Engel (1956) established bio- and subfacies in Texas bays and considered several environmental factors as important but did not select specific factors as being primary. Engel (1956) summarized the unpublished open marine study of Curtis (A.P.I. Project

51, Report 18) who established bio- and subfacies on the basis of depth. Puri and Hulings (1957) considered food supply as the most critical environmental factor controlling the distribution of ostracods along the west coast of Florida. Hulings (1958a;b) established open marine biotypes on the basis of the type of substratum and the composition of the living ostracods. Curtis (1960) found that ostracod biofacies agreed in general with sediment units on the Mississippi Delta. She concluded that "current pattern and depth appear to be the principal causal factors in esteblishing the environmental pattern." Waller (1961) concluded on the basis of studies in the St. Andrews Bay system that "the only clear animal-substrate relationship observed was between the ostracods and the sea grasses." He suggested that sediment-ostracod relationships were of secondary importance in that both were controlled by currents and found that salinity is quite important in the distributional pattern of faunal elements. Benda and Puri (1962) concluded that the distributional patterns of ostracods in the Cape Romano area of Florida did not appear to be controlled by one particular environmental factor, rather a function of a combination of factors. The authors stated that "striking relationships are observed between faunal distribution patterns and mean grain size of sediments, organic carbon content of sediments, salinity-temperature of the bottom water, and submarine topography." Hulings and Puri (1964) summarized studies along the west coast of Florida and proposed shallow water assemblages based entirely on the occurrence of live ostracods. Several environmental factors were evaluated and the authors concluded that no one factor controls the distribution of species rather a complex of factors, the primary being salinity, temperature and organic content. Morales (1965; 1966) evaluated several environmental factors in Laguna de Terminos, Campeche, Mexico and recognized three assemblages. He found that the most favorable environment was the area of water rich in submerged vegetation and a zone of turbid water poor in submerged vegetation.

Most of the ecological studies in the Gulf have been based on one sampling in an area and the ecological interpretations have been based largely on the distribution of dead ostracods with little or no regard to the living. There is, of course, a serious fallacy in this approach in that it has been demonstrated that living populations fluctuate considerably in certain areas and species diversity of dead populations is generally greater than diversity of living populations (Kornicker and Wise 1960b; Baker and Hulings 1966). Dead populations are subject to transport and concentration by currents. It is obvious, therefore, that if valid ecological studies are to be conducted, then the sampling must be for living ostracods and repeated over a sufficient period of time to take into account seasonal variation in physical, chemical and biological variables. Furthermore, it is difficult on the basis of the above ecological studies to properly evaluate the role of environmental factors in the distribution of ostracods. Individual factors or a complex of factors have been proposed. Until taxonomic problems are resolved, it will be difficult to utilize the ecological information that is available on much more than a local level instead of a regional level; *i.e.*, the entire Gulf of Mexico.

Seasonal Studies.—Field studies on the seasonal distribution of marine ostracods have been conducted by relatively few investigators. Hulings (1958a) presented limited information, based on one summer and one winter sampling, a tempera-

TABLE 1

Recent marine Ostracoda (excluding Myodocopa and Cladocopa) from the Gulf of Mexico with author, salinity, temperature, and depth range, sediment type and environment

Taxa	Author(s)[a]	Salinity (ppt)	Temperature (C)	Depth (m)	Sediment[b]	Environment[c]
Actinocythereis						
bahamensis (Brady)	11*	34.3–36.1	20	7.5	10	14
exanthemata (Ulrich and Bassler)	5*,6*,7,8,11*,13, 14,15,16,17*,18*	1.8–37.9	13.5–32	1–55	1,3,5,6,8,9,11, 12,13,15,16	2ac,3,5b,9,10,11,12, 13abc,15c,17,18,19
exanthemata marylandica (Howe and Hough)	7,13	. .	. .	. .	. .	. .
margaretifera (Müller)	6	. .	. .	15–22.5	. .	. .
subquadrata Puri	13*,14,15,16*,18*,24	27.4–34.4	27–32	1–2	1,3	7ab,17,18,19
Acuticythereis						
laevissima (Edwards)	13*,15,16*,18*	22.9–34.9	15–29.5	2–22	1,8	18,19
multipunctata Edwards	13*	. .	. .	. .	. .	. .
tuberculata Puri	13*,24	32	33	1	1	. .
Anomocytheridea						
floridana (Howe and Hough)	16*	. .	. .	. .	. .	16,18
Argilloecia						
cylindrica Sars	2,4,11*	34.3–36.1	20	5–823	10	14
minor Müller	11*	34.3–36.1	20	5	10	14
Aurila						
amygdala (Stephenson)	17*,25*	36.91–39.92	. .	7–25	1,7	. .
conradi (Howe and McGuirt)	13,15,16,18*	14.7–33.8	14.3–32.0	2–12	1,3,8,17	18,19
conradi floridana Benson and Coleman	17*	36.17–39.92	. .	6–44	. .	. .
conradi littorala Grossman	23*	6.0–65.0	6–36	. .	2	. .
conradi var A	8	0.7–28.0	28–31	1–5	3,4	9
conradi var B	8	25.0–36.3	14.8–30.2	1–52	1,5,15,16,17	10,11,12,13abc
floridana Benson and Coleman	19,25*	16–39	. .	. .	1	. .
Bairdia						
cf. *B. bradyi* Bold	17*,25*	36.28–39.92	. .	6–51	1	. .
coronata Brady	1*,4	. .	. .	. .	. .	. .
crosskeyana Brady	13*,16*	. .	. .	. .	. .	18,19

dewattrei Brady	1*	. .	. .	. .	. .	. .
foveolata Brady	13	. .	. .	. .	. .	. .
gerda Benson and Coleman	17*	34.86–39.92	. .	7–17	. .	. .
laevicula Edwards	16*	. .	. .	. .	. .	19
milne-edwardsi Brady	13*	. .	. .	. .	. .	. .
ovata Bosquet	2	. .	. .	1250+	. .	. .
shulerae Puri	13,16*,18*	>30	. .	<20	3	18,19
cf. *B. tuberculata* Brady	13*,16*	. .	. .	. .	. .	19
victrix Brady	2,13*,17*	36.17–39.36	. .	6–51	. .	. .
Bairdoppilata						
triangulata Edwards	17*	36.17–39.36	. .	6–80	. .	. .
Basslerites						
cf. *B. berchoni* (Brady)	11*	34.3–36.1	20	>5	10	14
minutus Bold	25*	30	. .	. .	1	. .
Bradleya						
hornibrooki Puri	13*	. .	. .	. .	. .	. .
Bythocypris						
bosquetiana (Brady)	2,4	. .	. .	1253–2523	. .	. .
compressa Brady	4	. .	. .	66–1920	. .	. .
laeva Puri	13*,15,16*,18*	22.9–35.9	13.5–29.5	2–16	1,8,17	17,18,19
Bythocythere						
turgida Sars	4	. .	. .	108–1372	. .	. .
Campylocythere						
concinnoidea Swain	5*,6*,11*,15,16,18*	18.1–37.9	15.3–30.5	2–20	1,6,8,10,13,17	5b,15c,19
laeva Edwards	7*,8,13*,15,16*,18*	27.7–35.8	15.0–30.1	2–20	1,8	10,13a,18,19
laevissims (Edwards)	7*,8,11*,17*	25.3–39.92	13.6–26.0	1–10	1,8,10	10,15b
multipunctata (Edwards)	7,8	23.3–35.3	23.6–30.2	1–8	5	11
Candona						
caudata Kaufmann	5*,6*	1.8	. .	1	1,6,10,11	2a,4,5a
lactea Baird	5*,6	1.8	. .	1	1,6,10,11	2ab,4,5a
marchia Hartwig	5*,6*,11*	0–2.8	20	2	1,6,8,10,11	2a,4,5a,15d
obtusa Bronstein	5*,6*	1.8	. .	1	1,6,10,11	1,2a,5ab

TABLE 1—Continued

Taxa	Author(s)[a]	Salinity (ppt)	Temperature (C)	Depth (m)	Sediment[b]	Environment[c]
Cativella						
cf. *C. runcinata* (Baird)	11*	34.3–36.1	20	to 30	10	14
Caudites						
angulata Puri	13*,16*	..	..	..	..	17,19
howei Puri	13*	..	..	..	..	..
Costa						
triplistriata (Edwards)	8	27.2–36.3	24.6–31.6	6–52	5,15	11,13b
Cushmanidea						
cf. *C. agricola* (Howe and Hadley)	11*	34.3–36.1	20	>30	10	14
anderseni (Puri)	8,11*,16*	25.3–36.1	24–25	to 30	1,6,10	13a,15b,17,18,19
ashermani (Ulrich and Bassler)	8	27.6–36.3	14.6–31.6	2–55	1,5,15,17	10,11,12,13b
cf. *C. echolsae* (Malkin)	11*	25.3–36.1	24–25	0–30	1,6,10	15b
elongata (Brady)	13*,15,16*,18*	22.9–33.7	11.2–28.2	2–16	1,5,8,17	17,19
selminuda (Cushman)	15,16*	22.9–33.1	27.1–30.0	2–8	1,8,17	18,19
cf. *C. ulrichi* (Howe and Johnson)	11*	34.3–36.1	20	>30	10	14
cf. *C. wilberti* Puri	11*	34.3–36.1	20	>30	10	14
Cyprideis						
castus Benson	22*,25*	18	..	..	1	..
floridana Puri	13*,16*,18*	..	..	..	3	18,19
littoralis Brady	5*,6*,8,15,18*	14.9–37.9	10.5–30.5	2–8	1,8,13,14,17	2b,5b,8
locketti (Stephenson)	5*,6*	1.8–36.1	..	1–2	1,6,10,11	2b,5a
mexicana Sandberg	22*,25*	25–30	..	..	1,10	..
ovata (Mincher)	22*	..	..	..	..	..
salebrosa Bold	21*,22*	..	..	..	..	..
torosa (Jones)	5*,6*,15,19	1.8–31.9	10.5–31.0	2–12	1,6,8,10,11,12	2b,5a
Cypridopsis						
vidua (Müller)	5*,6*	1.8	..	1	1,6,10,11	1,5a
Cyprinotus						
salinus (Brady)	5*	1.8–37.9	..	1–2	1,6,10,11,13,14	..

Cypris						
azteca Saussure	1	. .	. .	. .	. .	. .
Cythere						
caudata Brady	7,13	. .	. .	. .	. .	. .
danaiana Brady	1*	. .	. .	. .	. .	. .
margaritifera Brady	1*	. .	. .	. .	. .	. .
montezuma Brady	1*	. .	. .	. .	. .	. .
silicula Brady	1*	. .	. .	. .	. .	. .
Cythereis						
dictyon Brady	4	. .	. .	63–3475	. .	. .
echinata Sars	4	. .	. .	31–3157	. .	. .
erica (Brady)	4	. .	. .	25–3230	. .	. .
margaritifera Müller	2,4	. .	. .	29–265	. .	. .
rastromarginata (Brady)	4	. .	. .	150–1902	. .	. .
silicula Brady	2	. .	. .	24–190	. .	. .
stolonifera (Brady)	4	. .	. .	88–3246	. .	. .
Cytherella						
grossmani Benson and Coleman	17*	34.86–39.92	. .	9–76	. .	. .
harpago Kornicker	25*	27–30	. .	4	1,6	. .
lata Brady	4,11*,15,16*	22.9–36.1	13.5–32.0	2–1720	1,8,10,17	14,18,19
obtusata Müller	2,4	. .	. .	43–198	. .	. .
polita Brady	2,4,11*	34.3–36.1	20	29–132	10	14
scotica Brady	8	27.2–36.3	14.0–30.2	2–45	5,15	11,13b
Cytherelloidea						
floridana	15,16	22.9–34.7	13.5–29.5	2–16	1,8	18,19
cf. *C. leonensis* Howe	18*	>30.0	. .	<22	1	. .
sarsi Puri	13*,17*	35.61–39.92	. .	9–51	. .	. .
umbonata Edwards	8	27.2–36.3	14.0–30.3	3–52	5,15	11,13b
Cytheretta						
danaiana (Brady)	7*,8,11	25.3–36.1	24.3–30.3	1–55	1,5,6,10,15,16	10,11,12,13abc,15b
multicarinata (Swain)	8	19.5	27.5	2	. .	8
sahnii Puri	15,16*,17*,18	31.1–37.84	13.5–30.0	2–21	1,8	19
Cytheridea						
setipunctata Brady	1*	. .	. .	. .	. .	. .

TABLE 1—Continued

Taxa	Author(s)[a]	Salinity (ppt)	Temperature (C)	Depth (m)	Sediment[b]	Environment[c]
Cytherideis						
aff. *C. ashermani* Ulrich and Bassler	6	. .	. .	. .	. .	. .
aff. *C. rugipustulosa* Edwards	6	. .	. .	. .	. .	. .
silicula (Brady)	2,4	. .	. .	18–174	. .	. .
Cytheromorpha						
curta Edwards	5	. .	. .	. .	. .	. .
paracastanea (Swain)	25*	27–34	. .	4	1,6	. .
pascagoulensis Mincher	5*,6*,8,15,18*	1.8–29.1	15.0–31.5	2–8	1,6,8,11,12,17	5a,8
warneri Howe and Spurgeon	7*,8,13*,15,16,18*	22.9–36.3	13.6–30.0	2–55	1,3,5,15,16,17	11,13abc,17,18,19
Cytheropteron						
alatum Sars	4,8,11*	34.3–36.3	20.0–25.5	30–1920	10,15	13b
howei Puri	13*	. .	. .	. .	. .	. .
latum Müller	11*	34.3–36.1	20	>30	10	14
cf. *C. leonensis* Puri	11*	34.3–36.1	20	>30	10	14
mucronalatum Brady	4	. .	. .	174–3431	. .	. .
Cytherura						
acuticostata Sars	11*,15,16*	34.3–36.1	20	4–30	1,8,10	14,19
cf. *C. cornuta* Brady	16*	. .	. .	. .	. .	18,19
costata Müller	5*,6,7*,8*,13*,14	10.5–37.9	24.6–30.0	1–45	1,5,13,14	2c,8,9,11,13a
elongata Edwards	5*,6*,16,18*,25*	1.8–37.9	. .	1–4	1,3,6,10,11, 12,13,14	2abc,5b,17,18,19
forulata Edwards	5*,6*,7,8,11*,13*, 15,16,18*,25	12.8–36.1	13.5–30.2	1–32	1,5,6,8,10,13, 14,15,16,17	2b,5b,8,9,10,11,12, 13abc,15bc,16,17,18,19
gibba (Müller)	15	12.8–26.6	28.0–31.0	2	1,8,17	. .
johnsoni Mincher	5*,6*,7*,8,13*,14,15	1.8–39.92	13.5–32.0	1–44	1,5,6,8,11,12	2b,3,5ab,8,9,11, 17,18,19
johnsoni subsp. A	6	. .	. .	. .	. .	. .
lineata Brady	2,4	. . .	. .	31–1810	. .	. .
cf. *C. paradoxa* Müller	11*	34.3–36.1	20	>30	10	14
rara Müller	5*,6*,11*,15,16	1.8–36.1	24.0–31.0	1–30	1,6,8,10,11, 12,13,14,17	2b,3,5b,15b

radialirata Swain	5*,6*,25*	1.8–38	. .	1	1,6,11,12	2a,5b
swaini Bold	25*	. .	. .	. .	. .	. .
wardensis Howe and Brown	7,8,13*,15,16,18*	10.5–36.1	15.0–29.8	1–26	1,5,8,17	9,10,11,12,13a, 17,18,19
Darwinula						
aurea (Brady and Robertson)	5*,6*	1.8	. .	1	1,6,10,11	2a,4,5a
Echinocythereis						
echinata (Sars)	8	33.7–36.3	24.3–29.9	22–55	1,15,16	13abc
garretti (Howe and McGuirt)	17*	36.17–37.39	. .	22–80	. .	. .
margaretifera (Müller)	11*	34.3–36.1	20	>30	10	14
Eucythere						
declivis (Norman)	4,16*	. .	. .	40–1920	. .	19
triangulata Puri	8	34.8–35.1	25.4–26.0	7	1	10
Eucytherura						
complexa (Brady)	4,11*	34.3–36.1	20	20–1400	10	14
Haplocytheridea						
bassleri Stephenson	5*,6*,7*,8,11*, 13,15,16	7.7–37.9	14.8–29.8	1–26	1,2,5,7,10,14	5b,8,9,11,13a, 15bc,18,19
bradyi (Stephenson)	5*,6*,8,13*,14,15, 16*,18*,20*,25*	1.8–37.9	14.3–31.6	2–38	1,2,3,5,8,10,14	5b,8,9,11,13a, 18,19
gigantea Benson and Coleman	17*	35.61–37.41	. .	7–21	. .	. .
cf. *H. nodosa* Stephenson	13	. .	. .	. .	. .	. .
ponderosa Stephenson	11*,13,15,16,19	18.1–34.3	14.3–27.8	2–8	1,6,8,10	15bc,17,18,19
proboscidiala (Edwards)	17*,19	34.86–37.79	. .	7–23	. .	. .
setipunctata (Brady)	15,16,18*,20*, 21*,25*	23.2–38	10.6–30.0	2–16	1,3,8	17,18,19
waltonensis (Stephenson)	11*,15,16*	18.1–32.5	13.5–27.7	2–16	1,6,10,17	15c,18,19
Hemicythere						
amygdala Stephenson	8,13*,15,16,18*	22.9–36.3	15.3–30.0	2–52	1,15,17	10,13b,17,18,19
angulata Sars	15	18.1–29.4	16.1–30.5	1–4	1,8,17	. .
cf. *H. confragosa* Edwards	7	. .	. .	. .	. .	. .
conradi Howe and McGuirt	5*,6*,7*,12	1.8–65.0	6.0–36.0	1–2	1,2,6,10,11, 12,13,14	5b
cf. *H. convexa* (Baird)	11*	34.3–36.1	20	>30	10	14

TABLE 1—Continued

Taxa	Author(s)[a]	Salinity (ppt)	Temperature (C)	Depth (m)	Sediment[b]	Environment[c]
cymba (Brady)	11*,13*	18.1–32.5	22	0–14	1,6,10	15c
emarginata Sars	15	10.5–30.5	10.7–30.0	2–4	1,8,17	. .
aff. *H. laevicula* Edwards	6*	. .	. .	. .	. .	. .
postconradi Puri	13*,16	. .	. .	. .	. .	18,19
villosa Sars	15	25.6–27.7	27.9–28.0	2	1,17	. .
Hemicytherideis						
aff. *H. mayeri* Howe and Johnson	5*	29.8–37.9	. .	2	13,14	2c
Hemicytherura						
cranekeyensis Puri	13*,15,16*,18*,25*	28–38	15.2	4–22	1,3,6,8,17	18,19
cf. *H. howei* (Puri)	11*	34.3–36.1	20	>30	10	14
cf. *H. purisubrhomboidea* Edwards	11*	34.3–36.1	20	>30	10	14
sablensis Benson and Coleman	17*	37.27–39.92	. .	6–9	. .	. .
Hulingsina						
anderseni (Puri)	15	28.4–31.8	14.3–27.7	1–2	1	. .
ashermani (Ulrich and Bassler)	15,16*,17*,18*	32.3–37.39	27.0–27.3	1–80	1,17	19
rugipustulosa (Edwards)	25*	. .	. .	. .	. .	. .
sandersi Puri	9*,18*	. .	. .	. .	. .	. .
sulcata Puri	13*,14,15,16,18*	9.7–34.9	10.6–30.0	1–20	1,3,8,17	18,19
tuberculata Puri	9*	. .	. .	. .	. .	. .
ulrichi (Howe and Johnson)	15,16	27.4	32.0	2	1	18
Kangarina						
bradyi Puri	13*	. .	. .	. .	. .	. .
cf. *K. quellita* Coryell and Fields	11*	34.3–36.1	20	>30	10	14
Krithe						
bartonensis (Jones)	4	. .	. .	40–3367	. .	. .
producta Brady	11*	34.3–36.1	20	>30	10	14
tumida Brady	2,4	. .	. .	197–3630	. .	. .
Leptocythere						
bacescoi (Rome)	11*	18.1–32.5	22	0–14	1,6,10	15c
cranekeyensis Puri	13*	. .	. .	. .	. .	. .

paracastanea Swain	5*,6*,11*	1.8–37.9	24–25	1–30	1,6,10,11,12,13,14	2b,3,5ab,15a
cf. *L. porcellanea* (Brady)	11*	18.1–32.5	22	0–14	1,6,10	15c
yoni Puri	13*	. .	. .	. .	. .	. .
Limnocythere						
sanctipatricii Brady and Robertson	5*,6*,8,14	1.8–37.9	23.6–33.0	1.6	1,5,6,11,12,17	5ab,8,9,10,11,12
sanctipatricii var. A Swain	5*	. .	. .	. .	. .	. .
Loxoconcha						
anderseni Puri	7*,8	33.7–36.3	26.1–31.6	12–23	15	13b
australis Brady	5*,6*,7*,8,11*,13* 14,15,16,17*,18*	0.7–37.9	15.0–33.0	1–32	1,3,6,13,14	3,8,9,10,13a, 15bc,19
avellana (Brady)	4	. .	. .	20–387	. .	. .
caudata Puri	13	. .	. .	. .	. .	. .
dorso-tuberculata (Brady)	4	. .	. .	190	. .	. .
elegans (Brady)	2,4	. .	. .	16–82	. .	. .
guttata (Norman)	7*,8	35.7–36.3	24.3–31.6	16–55	1,15,16	13abc
impressa (Baird)	15	10.9–31.3	18.5–28.8	1–12	1,8,17	. .
cf. *L. laevis* Brady	16*	. .	. .	. .	. .	18
matagordensis Swain	5*,6*,7*,8,13*,14, 15,16,18*,25*	0.7–39	13.5–30.0	2.8	1,3,6,10,13,14	2c,5b,8,9,17,18,19
ochlockoneensis Puri	13*	. .	. .	. .	. .	. .
postdorsoalata Puri	13*,18*	. .	. .	. .	1,3,17	. .
purisubrhomboidea Edwards	11*,19,23*,25*	16.0–37.0	24–25	0–30	1,6,10	15b
reticularis Edwards	16*	. .	. .	. .	. .	19
sarasotana Benson and Coleman	17*,25*	27–37.84	. .	7–21	3	. .
subrhomboidea Brady	11*	25.3–34.3	22–24	0–14	1,6,10	15bc
wilberti Puri	7*,8,13*	33.7–36.3	24.3–31.6	12–55	15	13b
Loxocorniculum						
fischeri (Brady)	17*	36.33–37.39	. .	9–31	. .	. .
postdorsoalatum (Puri)	17*	36.23–39.92	. .	6–21	. .	. .
Luvula						
palmerae Coryell and Fields	11*,15,16	25.3–36.1	18.5–27.5	0–30	1,6,8,10	15b,17,18,19
Machaerina						
tenuissima (Norman)	11*	34.3–36.1	20	>30	10	14

TABLE 1—Continued

Taxa	Author(s)[a]	Salinity (ppt)	Temperature (C)	Depth (m)	Sediment[b]	Environment[c]
Macrocypris						
africana Müller	3*,4	..	..	20–22	..	..
decora (Brady)	2,4	..	..	68–210	..	..
maculata (Brady)	4	..	..	57–1720	..	..
schmitti Tressler	3*,4,13	..	..	..	..	..
similis Brady	2,4	..	..	33–144	..	..
tumida Brady	2,4	..	..	1400–1800	..	..
Megacythere						
johnsoni (Mincher)	18*,25*	..	..	..	6	..
robusta (Puri)	13*,18*	>30.0	..	<32	3	..
stephensoni (Puri)	13,15,16*,25*	26–39	13.5–32.0	2–12	1,6,8	17,18,19
striata (Puri)	15,16	22.9–33.9	14.7–32.0	2–16	1,17	17,18,19
Microcythere						
cf. *M. inflexa* Müller	13*	..	..	..	..	..
johnsoni Mincher	5*,6*,8,11*	0.7–37.9	22.0–33.0	0–14	1,6,10,13,14	2d,5b,9,15c
moresiana Stephenson	11*	25.3–36.1	24.0–25.0	0–30	1,6,10	15ab
stephensoni Puri	6*,8,11*	18.1–32.5	22.0–28.6	0–14	1,5,6,10	11,15c
Mutilus						
confragosa (Edwards)	13	..	..	..	..	..
Neocaudites						
nevianii Puri	13*,16*,18*,25	28–39	..	4	1,3,6	17,18,19
Nesidea						
cushmani Tressler	3*,4	..	..	4–22	..	..
ovata (Bosquet)	4	..	..	1810	..	..
victrix (Brady)	4	..	..	190–2395	..	..
Orionina						
bermudae (Brady)	7*,8,13*,15,16*,17*	34.8–39.92	15.7–28.3	7–52	1,15	13b,19
bradyi Bold	25*	38	..	..	1	..
Paijenborchella						
mediterranea Ruggieri	25*	..	..	..	1	..

Paracypris						
polita Sars	11*	34.3–36.1	20	>30	10	14
sablensis Benson and Coleman	17*	36.23–39.92	. .	6–21	. .	. .
Paracytheretta						
multicarinata Swain	5*,6*,15,19	27.1–37.9	11.7	2	1,2,6,10,13,17	2c,5b
Paracytheridea						
cf.*P. chipolensis* Howe and Stephenson	11*	34.3–36.1	20	>30	10	14
troglodyta Swain	5*,6*,8,11*	1.8–37.9	20.0–30.0	1–2	1,6,10,11,12,13,14	2abc,5ab,8,9,15d
tschoppi Bold	17*	35.61–39.41	. .	6–44	. .	. .
vandenboldi Puri	5*,8,15,25*	10.5–37.9	24.8–29.8	1–20	1,13,14	2c,8,9,10,13a
washintonensis Puri	8,13*,15,16*,18*	19.7–36.3	24.8–30.3	1–27	1,15	8,9,10,14,19
Paracytherois						
ensiforme (Brady)	11*,13,15,16	22.9–36.1	14.3–32.0	2 to >30	1,7,10	14,18,19
flexuosa (Brady)	2,4	. .	. .	43–190	. .	. .
Paradoxostoma						
atrum Müller	5*,6*,7*,13*	29.8–37.9	. .	2	13,14	2b,5ab
ensiforme Brady	2,4,5*,6,7,14,18*	27.1–36.1	. .	1–90	1,6,10	2d
Pellucistoma						
aff. *P. howei* Coryell and Fields	11*	25.3–36.1	24.0–25.0	0–30	1,6,10	15ab
magniventra Edwards	7*,8,13*,15,16*, 17*,18*,25*	22.9–39.92	14.3–32.0	2–31	1,5,6,8	11,13a,18,19
Perissocytheridea						
bicelliforma Swain	5*,6*,8,18*,25*	3.5–36.1	28.4–30.0	1–2	1,6,10	2d,5ab,8
bicelliforma var. *propsammia* Swain	5*	27.1–36.1	. .	2	1,6,10	. .
brachyforma Swain	5*,6*,8,11*,14,15, 16*,18*,25*	07–37.9	10.7–31.0	2–12	1,6,10,13,14	5ab,9,15bc
brachyforma excavata Swain	5*,6*	1.8–37.9	. .	1–20	1,6,10,11,12, 13,14	2ab,3,5b
excavata Swain	25*	18–30	. .	2	. .	. .
laevis Benson and Coleman	17*	36.17–36.87	. .	8–80	. .	. .
matsoni (Stephenson)	11*	0–28.9	20	2	1.8	15e
rugata Swain	5*,6*,8,16*,19,25	2.1–38	27.0–28.6	1–4	1,6,10	2d,5b,8,17
subrugosa (Brady)	21*	. .	. .	. .	. .	. .

TABLE 1—Continued

Taxa	Author(s)[a]	Salinity(ppt)	Temperature(C)	Depth (m)	Sediment[b]	Environment[c]
Physocypria						
pustulosa (Sharpe)	5*,6*,16*	1.8	. .	1	1,6,10,11	1,2a,4,5a,16
Platella						
mulleri Puri	13*,16*	. .	. .	. .	. .	18
Pontocypris						
intermedia Brady	3*,4,8,13	27.2–36.2	14.0–30.2	4–55	1,5,15,16	11,13abc
subreniformis Brady	4	. .	. .	29–1600	. .	. .
trigonella Sars	2,4	. .	. .	190–1417	. .	. .
Potamocypris						
smaragdina (Vavra)	5*,6*	1.8–28.9	. .	1	1,6,11,12	2a,4,5a
Protocytheretta						
danaiana (Brady)	10*,13*,15,17*,18*	33.0–3739	18.5	8–44	1,8	. .
multicarinata (Swain)	16*	. .	. .	. .	. .	18
Pseudocythere						
caudata Sars	2,4	. .	. .	310–1372	. .	. .
fragilis (Sars)	11*	34.3–36.1	20	>30	10	14
Pterygocythereis						
aff. *P. americana* (Ulrich and Bassler)	17*	36.17–39.41	. .	6–80	. .	. .
jonesi (Baird)	7,8,13*,15	22.9–36.3	14.3–30.3	2–55	1,5,8,15	11,13b
Puriana						
dawsoni (Brady)	11*	25.3–36.1	24.0–25.0	0–30	1,6,10	15ab
fissispinata Benson and Coleman	17*	36.27–36.82	. .	24–31	. .	. .
floridana Puri	13*,18*	>30	. .	<22	1,3,17	. .
rugipunctata (Ulrich and Bassler)	6*,7,8,13*,15, 16*,17*,18*	22.9–39.92	14.0–32.0	2–80	1,5,15,16	11,13abc,18,19
Reticulocythereis						
floridana Puri	13*	. .	. .	. .	. .	. .
multicarinata (Swain)	18*	. .	. .	. .	. .	. .
Sahnia						
aff. *S. subulata* (Brady)	11*	34.3–36.1	20	>30	10	14

Tanella						
gracilis Kingma	25*	25–38	. .	1–12	1,6,10	. .
Triebelina						
bradyi Triebel	13*	. .	. .	. .	. .	. .
coronata (Brady)	13*,16*	. .	. .	. .	. .	19
indopacifica Bold	13*	. .	. .	. .	. .	. .
sertata Triebel	13*	. .	. .	. .	. .	. .
Xestoleberis						
curta (Brady)	4,11*	33.4–36.1	24–25	30–265	1,6,10	15a
expansa (Brady)	2,4	. .	. .	150–3246	. .	. .
intermedia Brady	13*,15,16	31.1–32.3	15.0–27.9	2	1,17	17,18,19
minima (Brady)	4	. .	. .	68–210	. .	. .
parva Müller	11*	34.3–36.1	20	>30	10	14
punctata Tressler	3*,4,13*,15,16	23.6–30.7	13.5–27.9	2	1	17
rigbyi Morales	24*	18–38	. .	2–4	1	. .

- [a] 1—Brady (1870)
- 2—Rothwell (1949)
- 3—Tressler (1949)
- 4—Tressler (1954)
- 5—Swain (1955)
- 6—Engle (1956)
- 7—Puri and Hulings (1957)
- 8—Hulings (1958a)
- 9—Puri (1958a)
- 10—Puri (1958b)
- 11—Curtis (1960)
- 12—Kornicker and Wise (1960a)
- 13—Puri (1960)
- 14—Kane (1961)
- 15—Waller (1961)
- 16—Benda and Puri (1962)
- 17—Benson and Coleman (1963)
- 18—Hulings and Puri (1964)
- 19—Kornicker (1964)
- 20—Sandberg (1964a)
- 21—Sandberg (1964b)
- 22—Sandberg (1964c)
- 23—Grossman (1965)
- 24—Keith and Hulings (1965)
- 25—Morales (1965)
- [b] 1—Sand
- 2—Sandy silt
- 3—Sand-mud mixtures
- 4—Mud
- 5—Muddy sand
- 6—Silt
- 7—Silty sand
- 8—Silty clay
- 9—Clayey silt
- 10—Clay
- 12—Gyttja-clay
- 13—Gyttja-silt-sand
- 14—Shell marl
- 15—Carbonate
- 16—Gravel
- 17—Grass
- [c] Swain (1955)
    - 1—Fluvial biofaces
    - 2—Bay biofacies
        - 2a—Upper bay subfacies
        - 2b—Midbay subfacies
        - 2c—Lower bay subfacies
        - 2d—Marginal subfacies
    - 3—Open Gulf biofacies
- Engel (1956)
    - 4—Prodelta subfacies
    - 5—Bay biofacies
        - 5a—Estuarine subfacies
        - 5b—Lagoonal subfacies
    - 6—Open Gulf nearshore biofacies
        - 6a——0-15f subfacies
    - 7—Open Gulf offshore biofacies
        - 7a—30-45f subfacies
        - 7b—45-100f subfacies
- Hulings (1958a,b)
    - 8—Upper bay (estuarine) biozones
    - 9—Lower bay (estuarine) biozone
    - 10—Sandy biozone
    - 11—Muddy sand biozone
    - 12—Grass flat biozone
    - 13—Outer neritic zone
        - 13a—Sandy biozone
        - 13b—Carbonate biozone
        - 13c—Gravel biozone
- Curtis (1960)
    - 14—Offshore biofacies
    - 15—Inshore biofacies
        - 15a—Inner neritic or nearshore subfacies
        - 15b—Estuarine subfacies
        - 15c—Open lagoonal subfacies
        - 15d—Interdistributary subfacies
- Benda and Puri (1962)
    - 16—Marsh-river environment
    - 17—Lagoonal environment
    - 18—Mangrove island environment
    - 19—Open Gulf environment.

* Illustrations provided.

ture range of approximately 13 to 30 C. Certain species were designated eurythermal and others stenothermal. Kornicker and Wise (1960b) in a study of the seasonal distribution of living ostracods in Texas bays found ostracods more abundant during the summer months. In Redfish Bay, *Hemicythere conradi* formed 90% of the population during the summer months while during the winter *Loxoconcha* sp. made up 55% of the ostracod population. The abundance of the latter species remained approximately constant during the year according to the author.

Waller (1961) found three distinct groups of ostracods in St. Andrews Bay and vicinity on the Florida Coast as far as seasonal abundance was concerned. Waller designated certain species as polythermal, others as oligothermal and still others as eurythermal. *Bythocypris laeva* and *Hulingsina sulcata* were found to reach maximum abundance in October; the remaining eurythermal species exhibited a clearly defined peak of abundance in December.

Kornicker (1964), on the basis of field studies in Redfish Bay, found that living ostracods were more abundant during the summer months, when the areas had high temperature and carbon production and the salinity was normal marine, and that the populations were very similar in composition within the study area. Furthermore, seasonal environmental differences were found to be reflected by changes in absolute abundance and in percentage frequency of species. He inferred "that low winter temperatures may be a factor in decreasing ostracod abundance during winter months."

Laboratory Studies.—There have been few laboratory studies on life histories, environmental limits, etc. on marine ostracods. Kornicker and Wise (1960a) working with *Hemicythere conradi* determined salinity and temperature tolerance. It was found that the species survived a salinity range of 6 to 65 ppt and a temperature range of 6 to 36 C. They also determined that *H. conradi* exhibited a positive response to light. In terms of sediment preference, *H. conradi* was found to prefer silty sand to coarser sediments. Grossman (1965) documented the appendage morphology of *Aurila conradi littoralia* and *Loxoconcha purisubrhomboidea* in relation to substratum.

Other Studies.—The study of Sandberg (1964b) deserves special mention because of its significance in systematics and influence of environment. Sandberg pointed out that variation in noding and swelling is a phenotypic character found in a number of genera, especially brackish water forms. He suggested that the variation in ecological conditions in the brackish water environment is responsible for phenotypic variation in ornamentation. He also observed genotypic difference in ornamentation as an expression of sexual dimorphism in some brackish water species.

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INCIDENCE OF TRYPANORHYNCHAN PLEROCERCOIDS IN SOME TEXAS COAST SCIAENID FISHES

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ABSTRACT

The incidence and the degree of infestation with trypanorhynchan plerocercus larvae in sciaenid fishes from south Texas coastal waters was determined during the summer of 1958. Plerocerci were found in all estuarine sciaenids, but more stenohaline species examined from the Gulf of Mexico were not infested. Among infested species a high incidence was found in *Bairdiella chrysura* (44.6%) and in *Cynoscion nebulosus* (32%). The larvae were primarily *Poecilancistrium caryophyllum.* The plerocercus stage of *Diplootobothrium springeri,* from the black drum, *Pogonias cromis,* is reported for the first time. An unidentified species of *Pterobothrium* was found in *Menticirrhus americanus.* Infestation relates to age of fish and, presumably, to a change in diet that introduces an appropriate vector harboring the procerci.

INTRODUCTION

Both commercial and sports fishermen complain of the "wormy" fish caught in the various Texas bays. Most complaints have concerned fishes of the family Sciaenidae. The cause of these complaints was investigated by Chandler (1935a) and shown to be the presence of a plerocercal stage of a trypanorhynchan cestode, which he named *Otobothrium robustum.* The parasite was common in the dorsal muscle masses of *Cynoscion nebulosus* from Galveston Bay. Later, Chandler (1954) reported heavy infestations in *Pogonias cromis* and stated that *Poecilancistrium robustum* (Chandler), or a closely related species, occurs in other sciaenids, lutianids and serranids. Systematic information on the degree of infestation, the distribution of the worms, the host species and whether one or several species of cestode are involved is virtually non-existent.

This paper deals with our attempts to define the distribution and the identification of the plerocercoid stage of cestodes in some Texas coast fishes, particularly sciaenids. The study was conducted during the summer of 1958 in the coastal region from San Antonio Bay to the southern end of the Upper Laguna Madre.

MATERIALS AND METHODS

Fishes were collected by means of otter trawls, hook and line, gill nets and trammel nets. The fish were iced down as caught, frozen until examined, identified and measured.

To detect prelocerci, the fish were filleted and the major muscle masses carefully dissected. In nearly all cases where worms were found, they were visible upon filleting the host. The cysts were fixed in aceto-formol-alcohol. In a few instances the larval worm was removed from the cyst prior to fixing, but most were removed after fixation. Worms were stained with Erhlich's hematoxylin, alum cochineal, or Mayer's acid carmine for subsequent identification.

The stomach contents of most of the fishes were examined to provide some idea of their feeding habits.

RESULTS AND DISCUSSION

ENCYSTING SITE AND APPEARANCE OF PLEROCERCI

The larvae were found most often in the dorsal muscle masses adjacent to the vertebral column and in the vicinity of the vent. They also occurred in the muscles just behind the cranium, under the skin adjacent to the dorsal fin and in the muscles of the caudal peduncle. On several occasions the posterior half of the worm was found protruding from the peritoneal lining into the abdominal cavity, suggesting entry into the muscles by penetration of the intestinal and peritoneal wall.

The cestodes were elongate and whitish-opaque in color with a terminal bulbous enlargement (the blastocyst). The larva was encysted in the blastocyst. The worms from all sciaenids except *Menticirrhus americanus* ranged up to 130 mm in length, 2–3 mm in diameter through the appendix and 4–5 mm in diameter through the blastocyst. They are aptly referred to as "spaghetti worms" by local fishermen. The larvae from *Menticirrhus americanus* were entwined about the viscera and in the mesenteries, but not in the muscles. These worms were yellowish in color and quite large. The largest, taken from a fish measuring 174 mm standard length, measured 290 mm overall and was 3–4 mm thick through the appendix and 6–7 mm thick through the blastocyst.

Larvae from the black drum, *Pogonias cromis*, had the blastocyst enclosed in a well defined cavity (host cyst?). The cavity remained well defined after removal of the worm. One interesting feature was the presence of these, or similar cavities, in the musculature of about one-half the drum examined. Yet, less than 5% of the fish actually harbored the larvae.

SPECIES OF LARVAE FOUND AND THEIR DISTRIBUTION

Otobothrium robustum Chandler was emended to *Poecilancistrium robustum* by Dollfus in 1942. Goldstein (1963) reduced *P. robustum* (Chandler) to a synonym of *P. caryophyllum* (Diesing). We choose to follow Goldstein in treating the larvae of *Poecilancistrium*.

Larval cestodes were found only in sciaenid fishes. Several other species (3 *Lagodon rhomboides*, 3 *Peprilus paru*, 16 *Polydactylus octoenemus*, 3 *Choloroscombrus chrysurus*, 7 *Paralichthys lethostigma*, 2 *Conodon nobilis*, 3 *Orthopristis chrysopterus*, 32 *Mugil cephalus*) were examined with negative results.

It is apparent that infestation varies with the species of fish (Table 1). *Bairdiella chrysura*, *Cynoscion arenarius*, *C. nebulosus*, *C. nothus*, and *M. americanus* were moderately to heavily infested while *Leiostomus xanthurus*, *Micropogon undulatus* and *P. cromis* were lightly infested. It is believed that the infestation of one *Leiostomus xanthurus* out of 492 specimens examined represents an accidental infestation. Data for *Larimus fasciatus*, *Sciaenops ocellata* and *Stellifer lanceolatus* are inconclusive since the number of specimens examined was small.

The most heavily infested species, *Bairdiella chrysura*, also contained the highest average number of worms per infested fish (1.65), not considering *L. xanthurus*. As many as five worms were taken from a single fish. In the other host species we never observed more than three larvae per fish. The number of worms per infested fish is far lower than that reporetd for *Cynoscion nebulosus* by Chandler (1935a), in which 20–30 worms per fish were common. Whether the number of worms in fish from the southern part of the Texas coast is con-

TABLE 1

Percentage of sciaenid fishes infested with the cestode plerocerci, *Poecilancistrium caryophyllum*

Species	Total fish examined	Percentage infested	Average number of worms per infested fish
Bairdiella chrysura	140	44.6	1.65
Cynoscion arenarius	142	14.8	1.43
C. nebulosus	87	32.0	1.39
C. nothus	229	8.3	1.21
Larimus fasciatus	3	...	...
Leiostomus xanthurus	492	0.2	2.0
Menticirrhus americanus[1]	30	10.0	1.0
Micropogon undulatus	408	2.2	1.22
Pogonias cromis[1]	328	3.4	1.36
Sciaenops ocellata	5	20.0	1.00
Stellifer lanceolatus	7	...	...

[1] The plerocerci found in these species were not *P. caryophyllum*. See text.

sistently lower than in fishes from the northern part of the coast is not known. Chandler (1935b), however, reported sharp differences in the parasite infestations of fishes from different localities in Galveston Bay and attributed these differences to an absence of suitable vectors. Although Chandler's interpretation may be entirely correct, until a more complete knowledge of the life cycle of *P. caryophyllum* is obtained the conclusion is premature.

All of the larvae, except those from *M. americanus* and part of those from *P. cromis*, were *P. caryophyllum* (*See* Plate 1). Larvae from *M. americanus* are referrable to the genus *Pterobothrium* and those from *P. cromis*, not belonging to *P. caryophyllum*, were *Diplootobothrium springeri*. This apparently constitutes the first record of the plerocercoid of this cestode. The adult was described by Chandler (1942) from *Platysqualus tudes* (= *Sphyrna tudes*) (*See* Addendum).

INFESTATION OF FISHES FROM DIFFERENT LOCALITIES

Fishes were collected from San Antonio Bay, Mesquite Bay, Aransas Bay, the Gulf of Mexico off Port Aransas, and from the Upper Laguna Madre, Texas. In general, during the period of collection, salinities were about 10, 15, 20–25, 32–35, and 40–45 ppt, respectively, for the above mentioned areas. Percent infestation for several species in each of the areas are given in Table 2. In most instances the numbers of fish taken per area are insufficient for statistical conclusions. However, for *B. chrysura* and *C. nebulosus* the percent infestation is high for most of the areas. This could reflect either the euryhalinity and motility of these fishes or the presence and abundance of infesting vectors in each area. With our data and incomplete knowledge of the life history of these cestodes it is impossible to assess these possibilities. We can say only that *P. caryophyllum* occurs in a variety of host species and over a considerable range of salinities.

INFESTATION AS A FUNCTION OF HOST SIZE AND FOOD

Presence of plerocerci in sciaenids appears to be a function of age. Infestation most likely reflects, therefore, a change in food habits and consequent introduction of a parasitized vector into the diet of the fish (Figs. 1 and 2). This is most noticeable for *C. nothus*, *C. arenarius* and *M. undulatus* and, to a lesser extent, in *C. nebulosus*. The numbers of fish that we examined in different size classes are adequate for comparison in these species only. Two-year classes are likley represented for *C. arenarius* (Fig. 1) with the classes overlapping somewhere between

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PLATE 1

FIG. 1 *Poecilancistrium caryophyllum* plerocercus blastocyst. Represented in several species of sciaenid fishes except *Menticirrhus americanus* and *Pogonias cromis*. (see Text).

FIG. 2 Scolex of *P. caryophyllum* freed from blastocyst.

FIG. 3 Enlarged view of portion of proboscis of *P. caryophyllum* to show hooks.

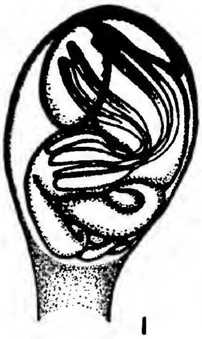
FIG. 4 Scolex of *Pterobothrium* sp. freed from blastocyst. In abdominal cavity of *Menticirrhus americanus*.

FIG. 5 Enlarged view of portion of proboscis of *Pterobothrium* to show hooks.

FIG. 6. Scolex of *Diplootobothrium springeri* freed from blastocyst. In muscle of *Pogonias cromis*.

FIG. 7 Enlarged view of portion of proboscis of *D. springeri* to show hooks.

110 mm to 130 mm standard length. In this species, the observed differences in infestation are considered to result from a change in food habits with age. Guest and Gunter (1958) indicate that the standard length of one-year-old fish is 170–180 mm. Our data for *C. nebulosus* (Fig. 1) show that infestation increases or starts at the end of the first year of growth. Accordingly, the percentage of fish



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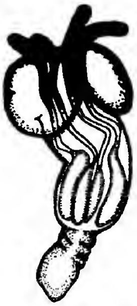

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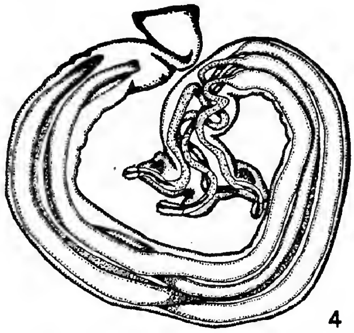

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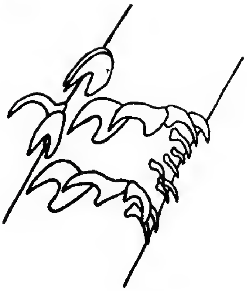

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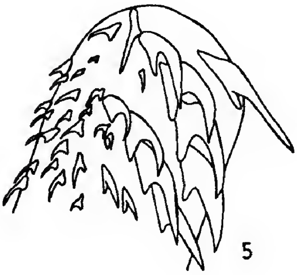

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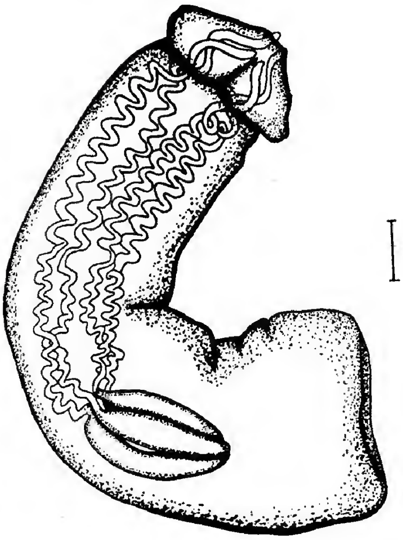

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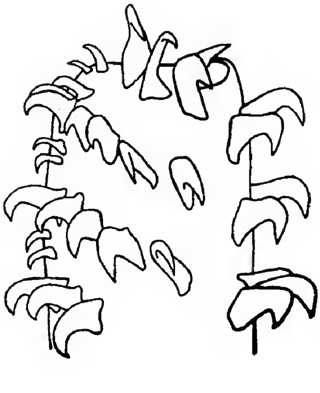

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TABLE 2

Percentage of sciaenid fishes infested with the cestode plerocerci, *Poecilancistrium caryophyllum* from various localities of the southern Texas coast.
Numbers in parentheses are total numbers of fish examined

Species	San Antonio Bay	Mesquite Bay	Aransas Bay	Gulf	Laguna Madre
Bairdiella chrysura	40(5)	37.5(16)	45.5(119)	....	....
Cynoscion arenarius	0(4)	0.0(1)	16.0(131)	0(6)	....
C. nebulosus	33(3)	0.0(5)	34.0(67)	....	33(12)
C. nothus	......	......	20.0(5)	8(224)	....
Larimus fasciatus	......	......	....	0(3)	....
Leiostomus anthurus	0(7)	0.0(46)	0.2(435)	....	0(4)
Menticirrhus americanus[1]	......	0.0(1)	8.0(12)	12(17)	....
Micropogon undulatus	0(26)	0.0(33)	2.6(348)	....	0(1)
Pogonias cromis[1]	......	......	0.0(1)	....	3.4(327)
Sciaenops ocellata	......	......	....	20(5)	....

[1] The plerocerci found in these species were not *P. caryophyllum*. See text.

infested rises abruptly in the second year, again suggesting that infestation relates to changes in food habits. Similar data were obtained for *C. nothus* (Fig. 1), *M. undulatus* (Fig. 2) and *R. chrysura* (Fig. 2). In the latter species, however, an insufficient number of small specimens were examined to be certain that very young fish are not infested.

The results strongly indicate that procercoid infestation occurs as sciaenids reach one year or more of age. Several workers have indicated that the diet of sciaenids is highly varied (Gunter 1947; Reid *et al.* 1956). Only Darnell (1958), however, directed his studies extensively to ontogenetic changes in diet. Although differences exist, Darnell found that two major diet-patterns occur. In very young sciaenids the primary food is composed of microplankton. A distinct shift to a varied crustacean diet occurs in sub-adult fish, which often shifts to a diet of fish and larger crustaceans in adults. Two of the major exceptions to this general feeding pattern occur in Texas Gulf Coast fishes. The black drum, *P. cromis*, shift almost exclusively to a shellfish diet as adults. The spot, *L. xanthurus*, seem to shift to a very mixed but mainly benthic diet (iliophagous). These and other studies state that the most common major element in the diet of young sub-adult and adult sciaenids of the Gulf coast are species of the commercial shrimps of the genus *Penaeus*.

The differences found in percent infestation for the various sciaenids (Table 1) emphasize the problem of whether each species of fish is a normal vector in the life cycle of the parasite or whether each species is accidentally infested by chance feeding. The high infestation for *B. chrysura indicates* that it is probably a common intermediate host. *C. nebulosus* and *C. arenarius* as well as *C. nothus* might be infested directly by the same vector as *B. chrysura* and therefore be normal intermediate hosts. The similarity in diet and feeding habits of these fishes are, perhaps, significant since they all are heavily infested with *P. caryophyllum*. That a common food, carrying the procerci, is the infesting vector, is a reasonable conclusion. But it is also possible that the various species of *Cynoscion* are infested by occasional feeding on infested *B. chrysura*. Although *B. chrysura* does not appear to be a preferred element in the fish diet of *C. nebulosus* (Guest and Gunter 1958; Gunter 1957) it was found during this study in the stomach of both *C. nebulosus* and *C. arenarius*. The sciaenids with low infestation possibly are infested by occasional feeding on a common vector carrying the procerci.

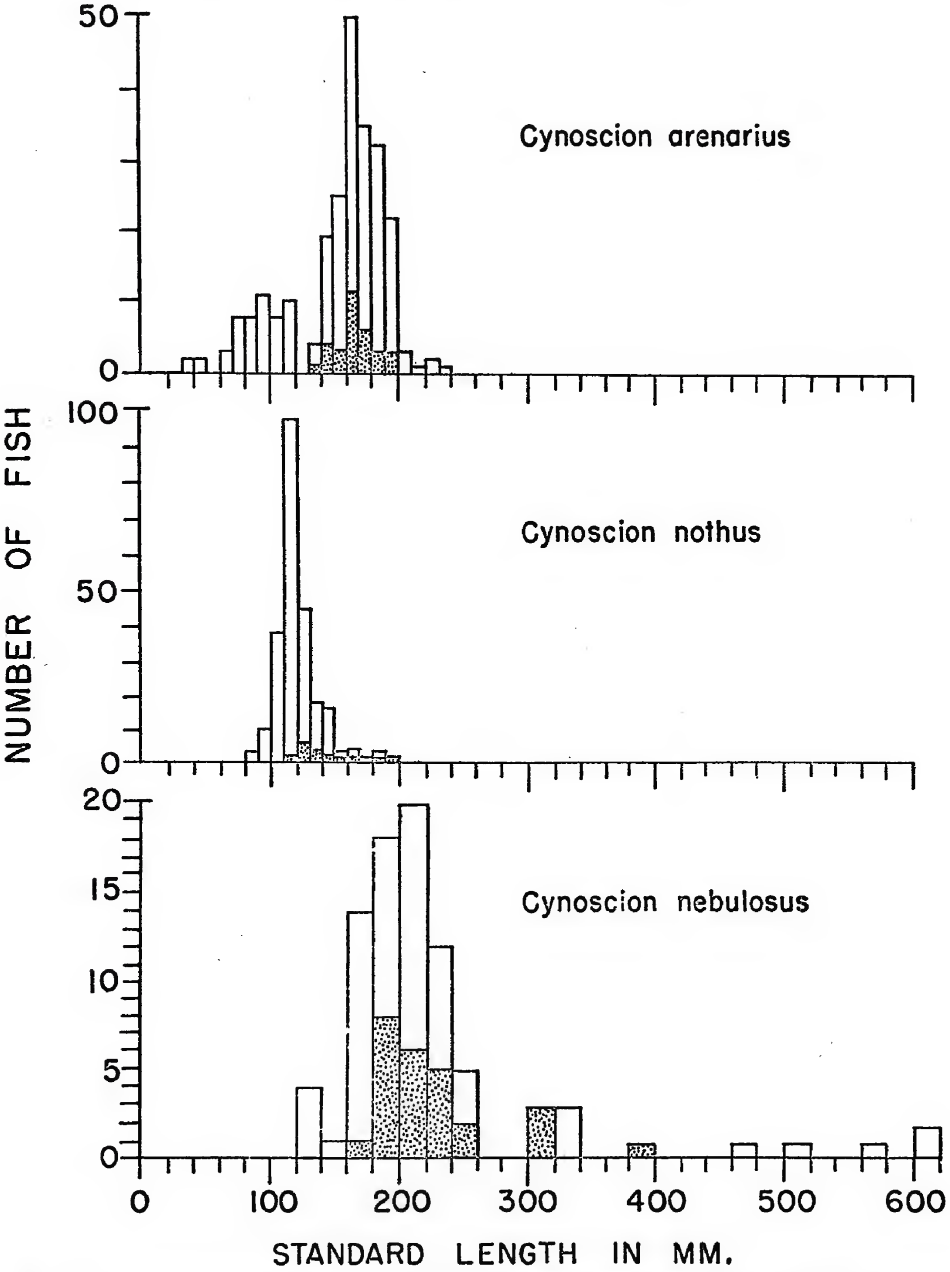

Fig. 1. Frequency distribution of specimens of *Cynoscion arenarius*, *C. nothus* and *C. nebulosus* and the number infested with the cestode *Poecilancistrium caryophyllum*. White bars are the number of fish per size group; black bars are the number of fish infested per size group.

The above reported results on age and incidence of infestation and the accumulated results of studies on the food habits of sciaenids do not answer the question of what common dietary item might harbor the procercoid larvae of *P. caryophyllum*. Commercial species of shrimp, as already suggested, are perhaps the most ubiquitous element in the diet of adult sciaenids in the coastal regions

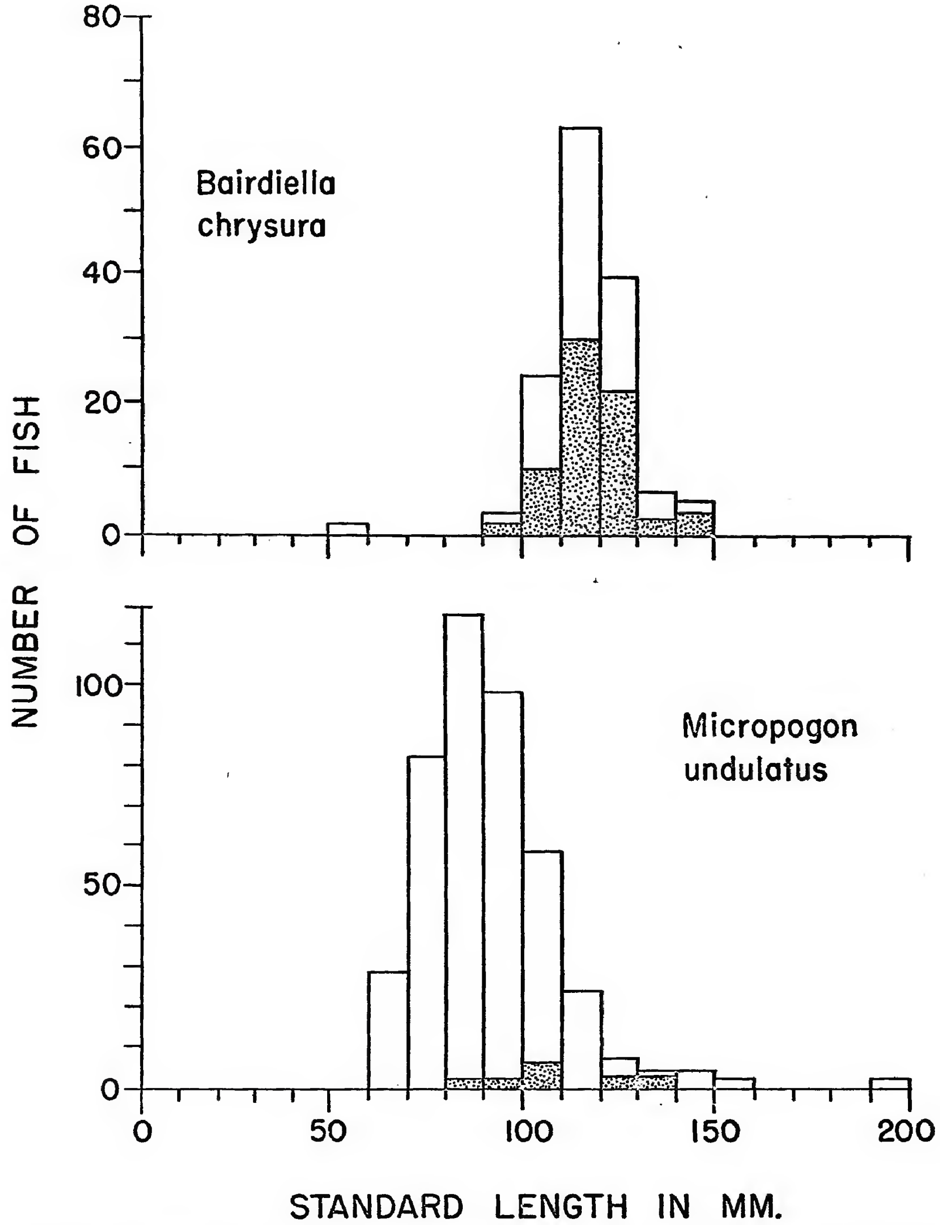

Fig. 2. Frequency distribution of specimens of *Bairdiella chrysura* and *Micropogon undulatus* and the number infested with the cestode *Poecilancistrium caryophyllum*. White bars are the number of fish per size group; black bars are the number of fish infested per size group.

of the Gulf of Mexico. Significantly, Sparks and Mackin (1957) have reported a larval trypanorhynchan (procerci) from the commercial shrimp *Penaeus setiferus* from Barataria Boy, Louisiana. But whether shrimp is the vector for sciaenids must remain speculative. The presence of trypanorhynchan larvae in shrimp and adult trypanorhynchans in elasmobranchs of the Texas coast, however, provide all the prerequisites for a complete life cycle.

PHYSIOLOGICAL PROBLEMS

The presence of the plerocerci of *P. caryophyllum* in various sciaenids poses the question as to whether the parasite shows adaptive plasticity, or, whether the milieux of the various host species are similar. In addition, the fact that many of the fish carrying plerocerci of *P. caryophyllum* occur in widely varying salinities suggests that either the life cycle of the parasites can occur at varying salinities or that the parasites are carried into the varied salinities by their hosts. Of the species of sharks in which the adult is found (Thatcher 1961; Goldstein 1962; 1963) only *Carcharinus leucas* enters water of salinity much below 25 ppt along the Texas coast, and then usually only during the summer months. If the eggs hatch in salinities ranging from 25 to 35 ppt and are carried by a crustacean (larval shrimp, etc.) it is possible that the plerocerci enter the bays with the yearly invasion of young crustacea, and are thus introduced into the fish population. The mass exodus of sciaenids from the bays into the Gulf waters during the winter months (Gunter 1947) could complete the life cycle by introducing the vector of the plerocerci into the diet of the more stenohaline elasmobranchs.

Such a cycle would imply that the physiological tolerances of the parasite are probably narrow and that the parasites are dependent on the ability of their respective hosts to maintain homeostatic balance in their tissues. Some of our data for sciaenids in hypersalinities are of particular interest in relation to this suggestion of limited salinity tolerances by plerocercoids. The vacuoles in the muscles of *P. cromis* may represent casts resulting from evacuation of the plerocerci (*P. caryophyllum*?) due to intolerable changes in host tissue constituents, such as inorganic ions, resulting from exposure to high salinities. This is emphasized by the fact that all but one of the *P. cromis* examined in this study were taken in the hypersaline Laguna Madre. Of these, only 3.4% were infested with plerocerci and they were species other than *P. caryophyllum*. Yet, approximately 50% of the black drum had the previously mentioned vacuoles in their tissues.

We cannot be certain, therefore, that the muscle casts in black drum represent cysts formed about plerocercoids that were subsequently resorbed. But under appropriate environmental conditions it is known that *P. caryophyllum* occurs in *P. cromis*. For instance, Chandler (1954) has reported heavy infestations of *P. caryophyllum* in *P. cromis* taken in Galveston Bay, where salinities are considerably lower than in the Laguna Madre. Surprisingly, *P. cromis* collected from the hypersaline Laguna Madre have been shown to retain both a high serum osmotic concentration and a high level of all major serum ions as compared to the very euryhaline striped mullet, *Mugil cephalus* (McFarland 1965). It is unfortunate that similar osmotic data are not available for *C. nebulosus* from a hypersaline environment, since this species, unlike *P. cromis,* does harbor *P. caryophyllum* when in the hypersaline Laguna Madre (Table 2). But it should be stressed that other physiological factors may be involved. Black drum, unlike *C. nebulosus,* feed almost exclusively on clams, at least when in the Laguna Madre. *C. nebulosis* feeds mainly on fish and crustaceans. The differences of infestation with *P. caryophyllum* between these two species and, particularly, the possible resorbtion of the pleurocercoid from the muscle tissue of black drum may be as much the result of differences in nutritional physiology as differences in osmotic regulatory capacity.

The fact that the parisite *P. caryophyllum* occurs in many species of sciaenids suggests that the physiology of the various hosts is similar. It is found primarily in one family of fish, the sciaenidae, in the Texas bays, but distinctively different members, like *Leiostomus xanthurus,* which has dark musculature, as well as different food habits, are not highly infested. However, Goldstein (1963) reported *L. xanthurus* as a host of the plerocerci of *P. caryophyllum,* but gave no data regarding incidence or intensity of infestation. Whether this represents an accidental infestation, as we consider our example to be, can be ascertained only when the major life cycle of *P. caryophyllum* is clearly established.

SUMMARY

The incidence of the plerocercus larvae of cestodes was investigated for several teleosts from Texas coastal waters. Infestation of several species of the family sciaenidae was heavy, individual species giving the following percentages: *Bairdiella chrysura*—44.6; *Cynoscion nebulosus*—32; *C. arenarius*—14.8; *Menticirrhus americanus*—10; *Cynoscion nothus*—8.3; *Pogonias cromis*—3.4; *Micropogon undulatus*—2.2; *Leiostomus xanthurus*—0.2. *Larimus fasciatus* and *Stellifer lanceolatus* had no parasites, but an insufficient number of specimens were examined to eliminate them as normal hosts. Of eight redfish, *Sciaenops ocellata,* three were found to be parasitized. Other fishes investigated were not infested with plerocerci. They were *Mugil cephalus, Polydactylus octonemus, Chloroscombrus chrysurus, Conodon nobilis, Lagodon rhomboides, Peprilus paru, Paralichthys lethostigma,* and *Orthopristis chrysopterus.*

Three species of trypanorhynchan cestodes were represented by plerocerci in the fishes examined: *Poecilancistrium caryophyllum, Diplootobothrium springeri* and *Pterobothrium* sp. *D. springeri* occurred only in *Pogonias cromis* and *Pterobothrium* sp. only in *Menticirrhus americanus. P. caryophyllum* was more widely distributed, having been found in all of the other sciaenids that were infested.

Infestation is related to age; younger fish were not infested. It is suggested that infestation occurs only with ontogenic food changes which introduce the vector into the diet. The most likely vector for at least many of the species is commercial shrimp. Data indicate that older fishes, particularly *Cynoscion nebulosus* and *C. arenarius,* are not as often parasitized as three-year old fish, suggesting a differential mortality of parasitized versus non-parasitized fish.

No significant difference in percentage infestation could be found for fish taken from different geographic and ecological localities in southern Texas marine

waters, but evidence is presented which suggests that plerocercoids may be resorbed in hosts that osmoregulate poorly when in hypersaline waters.

ACKNOWLEDGMENTS

This study was conducted while we were employed by the Texas Game and Fish Commission, Marine Fsheries Division. We wish to express our appreciation to the many members of this organization who gave freely of their time and knowledge. Special thanks are due to Mr. Howard T. Lee, former division director. The extensive fish collection could not have been made without the capable assistance of Sr. Angelo Flores, boat-captain, and Sr. Paul Dominguez, deckhand.

Drs. J. G. Mackin and S. W. Hopkins have contributed materially by criticizing the manuscript and making many helpful suggestions.

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ADDENDUM

Diplootobothrium springeri plerocercus

(Plate 1, Figures 6 & 7)

Description: Scolex very broad, round, and long (11 mm). Bothria two, 1.3 mm long and 1.5 mm wide, provided laterally with ciliated sensory

> pits. Width of head behind bothria 2.25 mm, nearly uniform for entire length. Proboscis sheath in broad spirals, 7 mm long. Retractor bulbs 2.3 mm long and 0.32 mm broad, the retractor muscles attached near the upper end. Proboscides about 0.15 mm broad exclusive of hooks, 0.25 mm broad including them. Hooks arranged in diagonal rows of 10 large, recurved, rose-thorn-shaped hooks, extending about two-thirds to three-fourths the distance around proboscis, the intervening area behind occupied by small hooks more or less irregularly arranged; some of the small hooks are only moderately curved and lack large bases. Largest hooks 100 microns long, with a base of similar length, smallest hooks 20 microns long.

The scolex of the larval stage does not differ greatly from the adult except in its generally smaller size. The main difference is that the adult scolex is flat whereas that of the larva is round. Otherwise the larva differs only by being reduced by approximately one-half in all dimensions. The proboscides of the larva were only slightly extended. Thus their true length could not be ascertained.

Host: *Pogonias cromis*—encysted in musculature.

Locality: Aransas Bay, Texas—additional specimens were obtained from the Upper Laguna Madre, Texas.

FATTY ACIDS IN SEA GRASSES AND MARSH PLANTS

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ABSTRACT

The seagrasses and marsh plants are important members of shallow marine ecosystems. The fatty acid composition of some of these plants was determined. None were found to differ much from the fatty acid patterns of terrestial plants, but definite differences in composition between plant parts were noted.

INTRODUCTION

Plants are the primary producers in the world's overall food chain. In the open sea, the algae are the carbon fixers. On land it is the grasses and other terrestial plants. These plants have long been studied, and much data on their fatty acid contents, though incomplete, are now available (Shorland 1962; Hilditch and Williams 1964).

Another type of ecosystem is the quiet shallow waters of bays and lagoons, where the primary producers are the so-called "seagrasses"; *i.e.*, the higher plants with root-like rhizomes found growing submerged in marine or brackish waters. Though there are relatively few species of these halophytes as compared to their fresh water counterparts, they occur widely and are found in nearly every shallow marine environment (Phillips 1960; Small 1933). *Thalassia testudinum,*

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Diplanthera wrightii (=*Halodule wrightii*), *Cymodocea manatorum* (=*Syringodium filiforme*) and *Ruppia maritima*, the most commonly found species in the Western Gulf of Mexico, were examined in this study. Also found with these, but not as abundant, are two species of *Halophila*, one of which is included here. Several of the marsh plants which grow along the edges and on the islands of bays were also studied.

Seagrass beds are essential for the support and continuation of many species of marine life, especially in their younger stages of development. They are the nurseries and feeding grounds for young fish and shrimp. Nutrients released from the dead and decaying grasses support a large plankton population and attract fish, worms, small crustacea and other such foragers as well as providing a rich substrate for bacteria. They also provide (physical) support for numerous epiphytic organisms which have a similar importance.

Furthermore, if such grass flats persist for any extended period, they can contribute to the subsequent geology and geochemistry of the area. For instance, in the Texas Laguna Madre, dense windrows of drifting *Diplanthera* and other grasses sometimes contribute to beach build-up when deposited on the windward shore. Also, cores of recent sediments in this same area have shown relatively well-preserved deposits of such grass more than 2000 years old (Behrens 1967—*pers. comm.*) If the grasses are supplying fatty acids to the sediment they might contain unusual fatty acids or fatty acid patterns which might help to identify this type environment in the geological record. Unfortunately, the patterns found were not diagnostic.

Since no report on the fatty acid composition of these grasses have been found in the literature, except that of Dudkin *et al.* (1964) who reported only formic, acetic, and propionic acids, this work may be considered as a preliminary survey. No effort has been made to prove the exact structure of the unsaturated acid by locating the double bonds, nor has the distribution of the fatty acids among the various lipid classes been determined, though this would undoubtedly be interesting and informative. A more extensive study may be possible at some later date.

MATERIALS AND METHODS

REAGENTS

Chloroform, methanol, 95% ethanol, hydrochloric acid, sodium and potassium hydroxides and chloroplatinic acid were all Baker Analyzed reagents. The methanol and chloroform were distilled from a glass still before use. CP grade boron trifluoride (BF_3) was obtained in lecture bottles from Matheson Company and sodium borohydride ($NaBH_4$)—99% purity from Delmar Scientific Labs, Inc. The activated charcoal used was Darco KB and the hexane was Matheson, Coleman and Bell Chromatoquality Reagent.

SAMPLES

Collections were from various places in Redfish Bay near Harbor Island between Aransas Pass and Port Aransas, Texas. Samples of *Spartina*, *Batis*, and *Salicornia* were taken from Harbor Island. All samples were either frozen or processed immediately.

ANALYTICAL PROCEDURE

The fresh or frozen plants were first washed with tap water to remove mud and loose debris and then with distilled water. They were immersed in dilute HCl for a short time to dissolve the carbonate deposited by attached organisms and to loosen other epiphytes. The plants growing above the water did not need the HCl rinse. The immersion in HCl was followed by a rinse with a stream of distilled water to flush away the loosened matter. Individual plants and shoots were separated by hand and rinsed again with distilled water to further eliminate contamination by any extraneous organisms.

Where possible, root, stem, and leaf parts were clipped to make up portions of differing plant parts (Gibbs 1947). The samples were then cut into manageable pieces with clean scissors and minced slightly with methanol in a mortar and pestle. The minced mixture was rinsed into a beaker with more methanol and sonicated with stirring for 15 minutes, after which the methanol was decanted and the solids sonicated in the same manner with chloroform. The filtered $CHCl_3$ was combined with the methanol and taken to dryness on a rotary evaporator. The residue was taken up in $CHCl_3$ and washed with 1N HCl to remove inorganic salts. This step was added after unusually high extract weights were noted in early samples. Unwashed extracts were found to contain from 85–93% inorganic salts. The $CHCl_3$ was then transferred to a tared container, evaporated under a stream of dry nitrogen and weighed to get the total lipid weight. The non-lipid plant material was weighed and added to the lipid weight to get the total dry plant weight. The total lipid was saponified with 0.5N KOH in methanol and the alkaline solution extracted with hexane to remove impurities. After acidification the fatty acids were extracted into chloroform and again evaporated under a dry N_2 stream. The methyl esters were prepared with 10% BF_3 in methanol, (Metcalfe and Schmitz 1961). The esters were taken up in hexane and transferred to a tared vial and weighed after evaporating the hexane with a dry N_2 stream. The esters were then redissolved in one ml hexane and analyzed by gas chromatography.

GAS CHROMATOGRAPHY

Columns—all 4 mm i.d. copper tubing:

1. 10% diethylene glysol succinate (DEGS) on 60–80 mesh acid-washed Chromosorb P (Johns-Manville) at 190 C.
    a.) for decanoic to eicosanoic—8.5-foot column with helium flow of 120 ml/min.
    b.) for palmitic to octacosanoic—6.0-foot column with helium flow of 155 ml/min.
2. 10% Apiezon L on 60–80 mesh Chromosorb Z (Applied Science Laboratories) at 210 C with a helium flow of 120 ml/min.

The DEGS columns were used for primary identification with the Apiezon L column used to confirm peaks in some samples. Peaks were identified by comparison to known standards and by linear-log plots of their retention times (Woodford and van Gent 1960). In other cases, 0.4 ml of the hexane solution was hydrogenated with sodium borohydride (Brown and Brown 1962) using a Brown[2] hydrogenator, and rechromatographed on the DEGS columns to see which peaks were due to unsaturated acids.

The instrument used in all cases was an F & M Biomedical Model 400 with a flame ionization detector.

Blanks using the same amounts of solvents and reagents were run with the samples. They showed less than 0.15 mg total fatty acids, mainly palmitic, palmitoleic, oleic and dodecanoic. Samples typically contained 1–9 mg total fatty acids as determined by GLC.

All results are reported as the weight percent of the total fatty acids detected.

RESULTS AND DISCUSSION

Lipid content of all samples ranged from 1.3 to 6.4% of the dry weight, essentially the same as in higher plants and some seagrasses (Garton 1960; Shorland 1963). Fatty acid contents are lower than Shorland (1961) reported for two typical land grasses, but are close to what has been found for sorghum (Burnett and Lohmar 1959). The amount of fatty acids weighed as methyl esters (0.4 to 3.9% of dry weight) was always greater than the amount detected by gas chromatography (0.1 to 1.2% of dry weight) indicating that an appreciable amount of non-acid lipid was carried through the isolation procedure. This lipid was usually about twice the amount of detectable fatty acids, though in one instance (*Ruppia*) there was nearly ten times more.

The fatty acid compositions of the seagrasses (Table 1) show no marked differences from those of the marsh grasses (Table 2) or from terrestial grasses

TABLE 1

Percent composition of the fatty acids of marine grasses

Plant	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3	>19
Ruppia maritima[1]	2.9	1.0	17.	5.2	1.9	6.0	26.	25.	9.0
Cymodocea manatorum[2]									
leaves	1.2	0.6	30.	4.9	1.8	4.8	23.	31.	1.9
rhizome	0.9	1.6	26.	4.0	1.6	14.	39.	6.0	5.2
whole	1.0	1.2	29.	4.4	1.5	7.2	30.	25.	..
Diplanthera wrightii[3]									
leaves	1.4	0.7	21.	3.4	2.4	5.5	24.	38.	..
rhizome	1.5	1.2	24.	2.9	2.8	13.	34.	5.0	14.
whole	0.9	0.6	25.	3.4	2.8	7.0	30.	28.	..
Halophila sp.[4]	1.5	0.5	27.	4.3	2.4	6.0	23.	29.	1.9
Thalassia testudinum[5]									
leaves	0.6	0.8	26.	2.2	2.2	5.7	27.	32.	2.1
rhizome	1.8	2.6	25.	6.3	3.7	24.	32.	4.4	..
whole	1.3	1.0	31.	3.0	4.1	9.5	19.	25.	..

[1] Contained also 0.8—br 17, 1.0—17:0, 1.2—17:1, 1.2—16:2, peak P—1.6, in percent.
[2] Contained also 1.0—16:2, 1.0—17:0, 0.8—17:0, 0.7—17:1, in percent.
[3] Contained also 2.0—br 17, 0.8—17:0, 0.9—17:1, in percent.
[4] Contained also 3.3—17:0, peak S—1.4, in percent.
[5] Contained also 1.0—br 14, 2.0—br 17, 1.4—17:1, peak S—3.3, in percent.

TABLE 2

Percent composition of the fatty acids of marsh plants

Plant	Total lipid	<14	14:0	16:0	16:1	18:0	18:1	18:2	18:3	>19
Salicornia perennis[1]										
leaves	6.4	tr	1.0	29.	1.2	1.3	9.6	15.	41.	. .
stems	4.9	0.8	1.4	27.	4.4	1.9	14.	27.	8.8	15.
roots	3.4	1.1	2.0	31.	4.2	2.6	17.	36.	4.8	. .
whole	5.1	1.2	1.4	29.	1.7	2.4	11.	18.	34.	. .
Salicornia bigelovii[2]										
leaves	5.4	0.5	0.5	25.	0.9	1.5	8.0	15.	48.	. .
roots and stems	3.9	0.6	1.5	28.	2.8	2.1	11.	25.	9.7	19.
Batis maritima[3]										
leaves	2.8	0.6	1.7	21.	2.6	3.3	7.2	21.	36.	. .
roots and stems	2.4	2.7	3.9	25.	6.2	3.4	11.	20.	13.	15.
whole	2.3	1.4	2.6	22.	2.9	3.0	7.8	20.	31.	5.7
Spartina alterniflora[4]										
leaves	1.5	0.6	1.5	25.	1.6	3.4	8.6	21.	32.	1.3
roots	1.3	3.9	1.4	23.	3.5	2.3	14.	31.	16.	3.0
whole	4.5	0.5	1.2	32.	2.9	4.3	12.	25.	18.	2.1

[1] Contained also, leaves 1.7—peak S, roots 1.0—15:0; whole 1.5—peak S, in percent.

[2] Contained also, leaves 1.4—peak S, 0.9—br 17, in percent.

[3] Contained also, leaves 0.5–15:0, 2.4—peak S, 1.4—br 17, 1.6—17:0, 0.9—peak P; roots and stems 1.7–15:0; whole 0.6—15:0, 1.8—peak S, 1.1—17:0, in percent.

[4] Contained also, leaves 0.9—15:0, 0.9—peak S, 0.6—br 17, 1.2—17:0, 1.0—peak P; roots 1.7—15:0; whole 1.5—15:0, 1.2–17:0, in percent.

referred to above. Palmitic, 18:3, and 18:2 are the major acids in the whole plant. (The number before the colon is the number of carbon atoms, and that following the colon is the number of double bonds.) However the roots and stems contain considerably less 18:3 than the leaves. Crombie (1958) noted a similar pattern in comparing green leaves to white or yellow leaves in several plants. The significance of this enrichment of green parts in 18:3 is not clear. There is a tendency for 18:2 and 18:1 to be concentrated in the roots and stems, while 16:0 is evenly distributed. The plant parts are further differentiated in that the leaves contain slightly more extractable total lipid and fatty acid than do the roots. The roots and stems, however, account for almost all of the fatty acids greater than 18 carbons in length. Traces of both branched-chain and odd carbon number acids were found in most samples but they are not reported unless they are at least 0.5%. Two unidentified peaks found in several samples are labeled "S" and "P." On DEGS, S elutes after 16:1 but before 16:2, and P elutes before 18:0. It could be a branched chain 18, but log-plot agreement is poor (*See* Fig. 1). It is interesting to note that both of these acids seem to occur only in the leaves of the plants examined.

In summary, the marine grasses and marsh plants of these typical coastal communities do not contain high concentrations of any unusual fatty acids. They are rich in the polyunsaturated 18 carbon acids. Because these plants are so abundant, their acids may be taken as typical for the type community they represent.

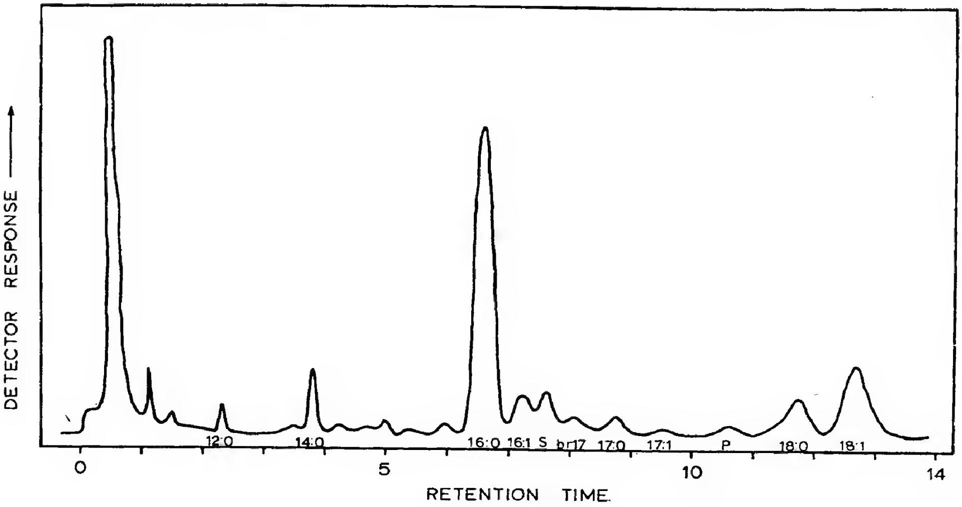

FIG. 1. Partial gas-liquid chromatogram of the methyl esters of the fatty acids from *Batis maritima* leaves. Column conditions: 8 ft × 4 mm; 10 % DEGS on 60–80 mesh acid-washed Chromosorb P; 120 ml/min helium; isothermal at 192C. br = branched chain, S and P = unidentified peaks, number before colon = chain length, number after colon = number of double bonds.

ACKNOWLEDGMENTS

Mr. Frank Moseley aided in collecting and identifying the seagrasses and made several helpful suggestions. Mr. Richard A. Hoover collected and identified the marsh plants.

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FORMS OF THE DINOFLAGELLATE *GYMNODINIUM BREVE* DAVIS IN CULTURES

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ABSTRACT

Morphological features of forms of the marine dinoflagellate *Gymnodinium breve* that were observed in cultures are described. Most forms are illustrated with photographs. Culture conditions at the times that various forms were observed are given. The most common encystment stage is described and compared with other forms. General features of cell division and other possible reproduction stages are illustrated and discussed. Forms resulting from variations in physical factors and reproductvie stages are discussed.

INTRODUCTION

Blooms of the marine dinoflagellate *G. breve*[2] and associated fish-kills have occurred sporadically in the Gulf of Mexico since 1946 (Wilson 1966). Toxic blooms of this organism have probably occurred since much earlier times. Although *G. breve* has been the subject of several investigations, very little is known of its morphology or life history.

Cultures of *G. breve* were first established in 1953 (Wilson and Collier 1955) and have been maintained continuously since then. The initial culture was isolated from a sample collected near St. Petersburg, Florida. Other isolations have been made from samples collected in the neritic zone between Clearwater, Naples and Alligator Point, Florida; and near Port Isabel and Galveston, Texas. Studies of cultures gave many opportunities to observe forms of this organism. Many forms would not be recognized as *G. breve* in field samples. For this reason, the following account attempts to describe the forms of this organism observed in cultures.

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[2] This dinoflagellate was originally described by Davis (1948) as *Gymnodinium brevis*. Since the specific ending did not agree grammatically with the generic ending, the specific name was changed to *breve*. This change required no nomenclatural justification.

Generally, culture conditions were similar to those previously described (Wilson and Collier 1955; Aldrich 1962; Aldrich and Wilson 1960; Wilson 1966). Some observations were made with bacteria-free culture, others with unialgal cultures.

GENERAL MORPHOLOGY

The absence of a cell wall permits considerable variation in the size and shape of *G. breve*; probably no two individuals are alike. Although many specimens observed in cultures or field samples could be identified from the original description (Davis 1948), the morphological type most frequently observed differed somewhat from his description. For this reason, the most frequently observed morphological type of cultures are described.

In sulcus view, the breadth of a typical, mature *G. breve* cell exceeded the length. The width of the hypocone, measured slightly below the girdle, was as much as 8μ greater than the measurement from the apex to the posterior margin of the hypocone (Fig. 1). The girdle was slightly anterior to the mid-line of the cell and was either displaced slightly to the left or none at all. The girdle and sulcus were well developed and the sulcus extended forward of the girdle almost to the margin of the epicone on the right side of the apex (sulcus view). Thus, the cell was divided into quadrants on the sulcus side. A crease extended from the sulcus at a position just anterior to the girdle toward the anterior left margin of the epicone. This crease and the sulcus extended along either side of an apical process or ridge, thus giving the cell a fifth division on the sulcus side. The cell was deeply cupped on the sulcus side with the maximum depression at the junction of the sulcus and girdle.

The broad side of the cell opposite the sulcus was slightly convex. The girdle and the hypoconal extension of the sulcus were the only prominent external features. The organism usually came to rest with this side down; making this surface viewed best with an inverted microscope. The hypocone was notched at the posterior position of the sulcus and the anterior margin of the epicone was usually depressed and slightly notched on either side of the apical ridge.

The thickness of a typical cell was slightly less than ½ the breadth (Fig. 1). Thus, a cell of 30 μ across the hypocone might be 12 to 13 μ thick. Although the apical process is seen best from a lateral view, the cell is difficult to observe at rest in this position. A cell moving away from the viewer appeared almost rectangular with a restriction at the sulcus.

In cultures, most mature cells were between 26 and 38 μ across the hypocone, 24 to 33 μ long and 12 to 16 μ thick. Immature or abnormal cells could be larger or smaller than these sizes.

Normally, *G. breve* had two flagella. The transverse flagellum was in the girdle and circumscribed the cell. This ribbon-shaped flagellum moved in an undulating motion and was the main locomotive organelle. The longitudinal flagellum was partly in the sulcus. It was whip-shaped and revolved when the cell was moving rapidly, and trailed almost without motion when the organism was moving slowly. It was the main maneuvering organelle and extended behind the cell *ca* 1.5 cell-lengths.

The cell membrane was smooth, clear and plastic. It was delicate and ruptured easily. When normal cells were placed in unfavorable conditions (*e.g.*, under a cover slip on a microscope slide), the sulcus, girdle and other external features gradually disappeared.

G. breve has a large, almost spherical nucleus, usually positioned in the right lobe of the hypocone (sulcus view). Its diameter was *ca* $1/3$ the breadth of the cell. It contained spiraled chromatin threads in a parallel arrangement and generally resembled the usual Dinophyceae nucleus (Dodge 1963).

Cells contained from one to more than 20 large chromatophores. Generally,

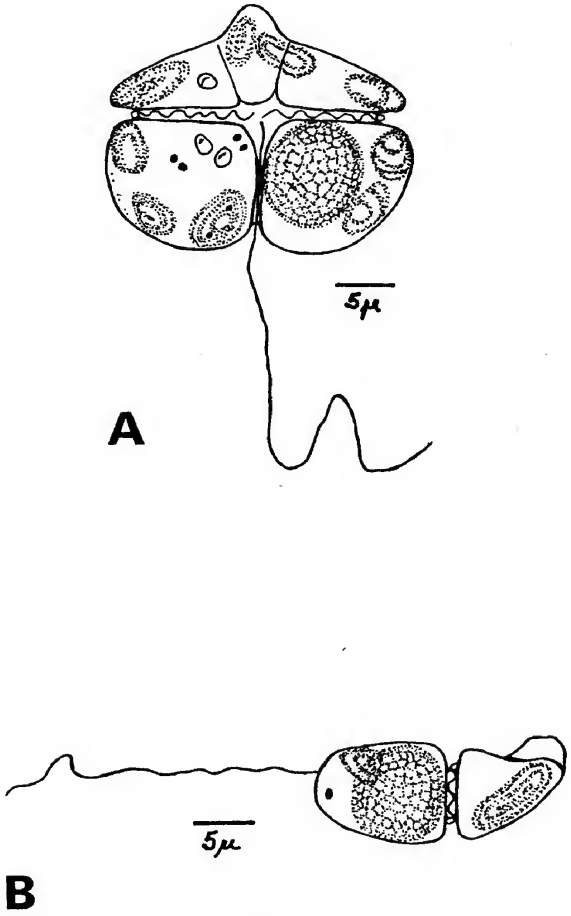

Fig. 1. Sketch of a typical, mature *G. breve* cell.

there were less than ten. The size of chromatophores was inversely related to the number present. The chromatophores were usually light-green or yellowish-green. Frequently, small dark-green granules could be observed which were either parietally positioned or scattered throughout the cell.

Highly refractive bodies were frequently observed (Fig. 2A). These were possibly oil droplets stored as food reserves. Such inclusions seemed to occur most frequently in mature cells. Opalescent colorless bodies were observed consistently in cell (Fig. 2B).

ATYPICAL CELLS

Atypical forms of *G. breve* occurred frequently in cultures. The extent of such variations in size and shape was difficult to determine because of the organism's extreme sensitivity and rapid response to external stimuli.

Laterally extended cells were observed occasionally in cultures (Fig. 2). The maximum cell size across the hypocone was *ca* 42 μ. These cells were possibly fully matured cells just prior to cell division. If a cell increases in size until division occurs and if some factor required for cell division is not available, cells may continue to increase in size without dividing. Finucane (*pers. comm.*) identified *G. breve* of field samples that measured over 90 μ across the hypocone. Similarly, Dragovich found forms of various breadths between 30 and 90 μ. According to them, these large forms occurred at a location 20 to 60 miles offshore during non-bloom periods. Studies of these large forms in cultures are needed to verify their identity.

A form of *G. breve* observed commonly in cultures was reduced laterally (Fig. 2C). The measurement across the hypocone was less than the measurement from the apex to the posterior of the hypocone. This form had a rounded epicone; the girdle was poorly developed and the sulcus was either poorly developed or absent. Some of these may have been daughter cells from recent cell division or their morphological features may have resulted from adverse culture conditions; *e.g.*, insufficient light (a continuous illumination of less than 100-foot candles—Fig. 3). This form of *G. breve* was commonly observed in field samples during bloom

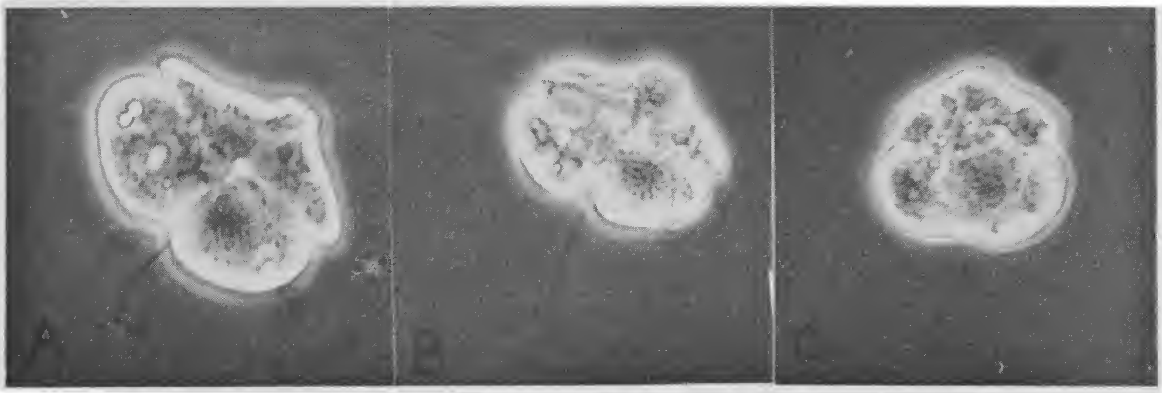

FIG. 2. Laterally expanded cell with refractive cytoplasmic inclusions (A); opalescent cytoplasmic inclusions along girdle on left side of cell (B); and a laterally reduced cell (C) (×480).

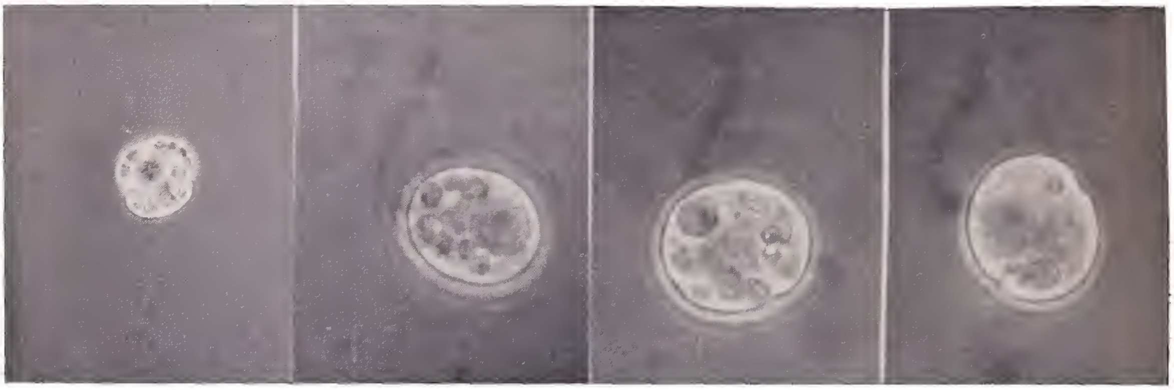

FIG. 3. Cells with altered sizes and shapes after periods in sufficient light (Cell on right—×240, others ×480).

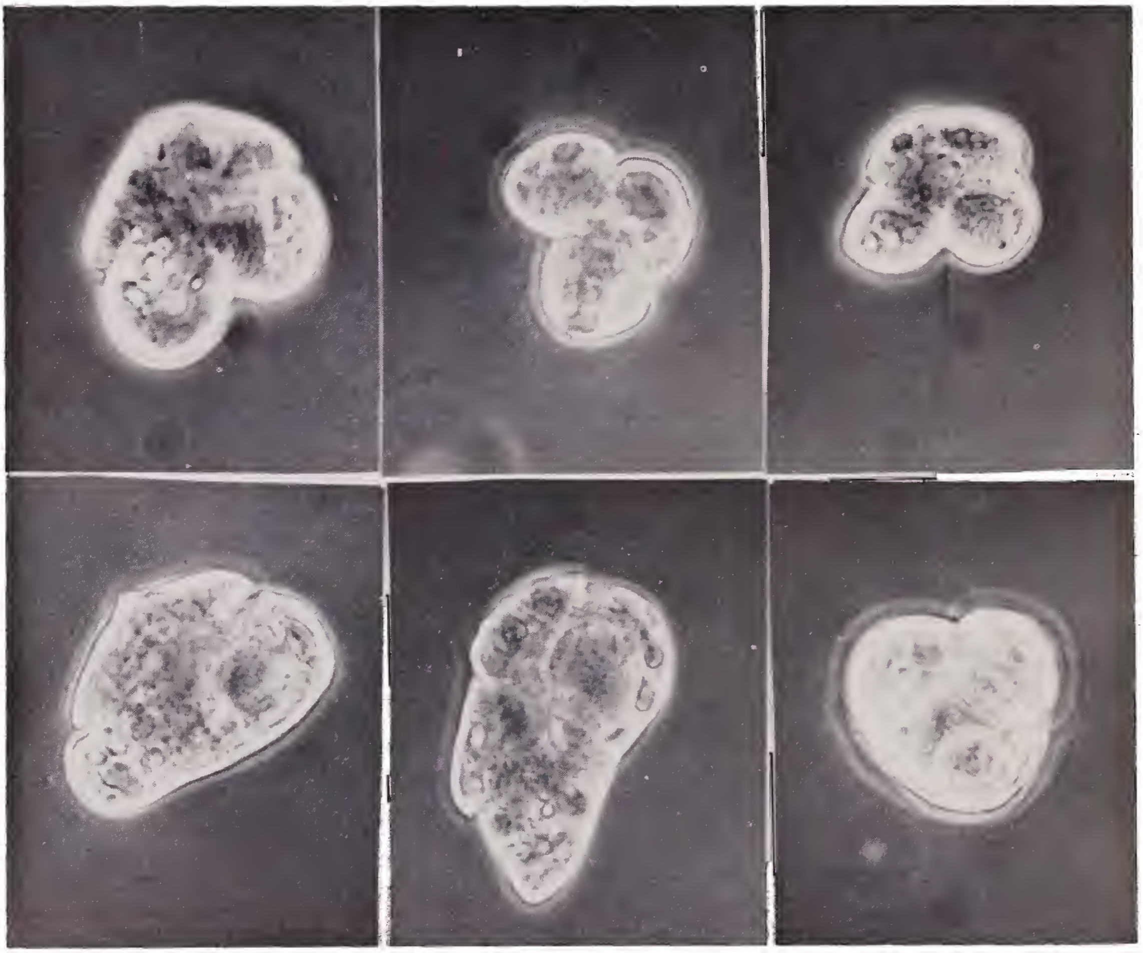

FIG. 4. Irregularly shaped *G. breve* cells (×480).

periods. It is possible that these small forms resulted from abnormally rapid cell division in contrast to excessively large forms resulting from a lack of cell division.

Periods of 3 to 5 days without light or longer periods with low light intensities (*e.g.*, 100-foot candles for 15 days) resulted in a gradual alteration in the size and shape of the cells. Cultures placed in the dark became spherical and smaller in 3 to 5 days (Fig. 3). The cells at this time measured 15 to 20 μ from apex through hypocone and 12 to 20 μ across the hypocone.

On some occasions, many irregularly-shaped cells appeared in cultures. A generalized description of these aberrant forms was not possible because of the lack of common features (Fig 4). Frequently, they were non-motile or had poor motility. As a result, they accumulated on the bottom of the vessel. Such cells lived 8 to 10 weeks in this condition when transferred individually with a micropipette to fresh medium. Some reverted to normal cells. The condition of some of these cells suggested that they might have been undergoing abnormal and incomplete cell division.

Cells subjected to high temperature, low salinity or other adverse conditions became less motile and sloughed the flagella. Later, they became spherical or ellipsoidal and their external features disappeared (Fig. 5). The cell membrane then became distended at one or more places. This distended portion was filled with an opaque fluid (Fig. 6) and continued to enlarge until the cell membrane ruptured.

An activity of *G. breve* frequently observed in natural populations, but rarely in cultures, was the flexing of the lateral portions of the cell along the sulcus. Although no experiments were conducted to verify phagotrophism, this activity suggested a possible mode of ingestion of minute food particles.

IMMOTILE FORMS

Three immotile forms that may be *G. breve* resting stages were observed in cultures. On the basis of their response to culture medium, two forms were viable; the other was non-viable.

The external features of the "non-viable" form were essentially that of a normal cell. The cell membrane was intact and had the size and shape of a normal cell. On the other hand, the cell possessed no detectable organelles. The cell membrane was filled with a homogeneous light-green material. There were no chromatophores. This form usually occurred in bright-green masses on the bottom of the culture vessels. The cells were always packed tightly together in a clear matrix. This condition and its repeated occurrence suggested that these cells formed aggregates and then became non-motile. For this reason, they are not believed to be a mass of cells that became aggregated by chance after dying. Cells of this type did not revert to free-swimming cells when placed individually in fresh culture medium by means of a micropipette.

The resting form most frequently observed was either spherical or ellipsoidal (Fig. 7). The organelles were frequently in a polar or parietal position. Most of these cells became spherical and their motility was reduced. The flagella were not sloughed, rather they became progressively shorter, as if they were resorbed by the cell. As a result, they retained some minor degree of motility during encystment. Encystment occurred only after the cultures approached maximum population levels—generally in excess of 2 × 10^3 cells/ml. Cysts usually occurred in yellow or greenish-yellow masses on the bottom of the culture vessel and in a position that received the most light. They did not withstand dessication. High temperature (35 C) and low salinity (15 ppt) caused these cysts to cytolyse; however, their appearance changed less after shaking for 2 hours than did normal cells. This type of cyst often reverted to a normal cell after it was transferred individually by means of a micropipette to fresh culture medium.

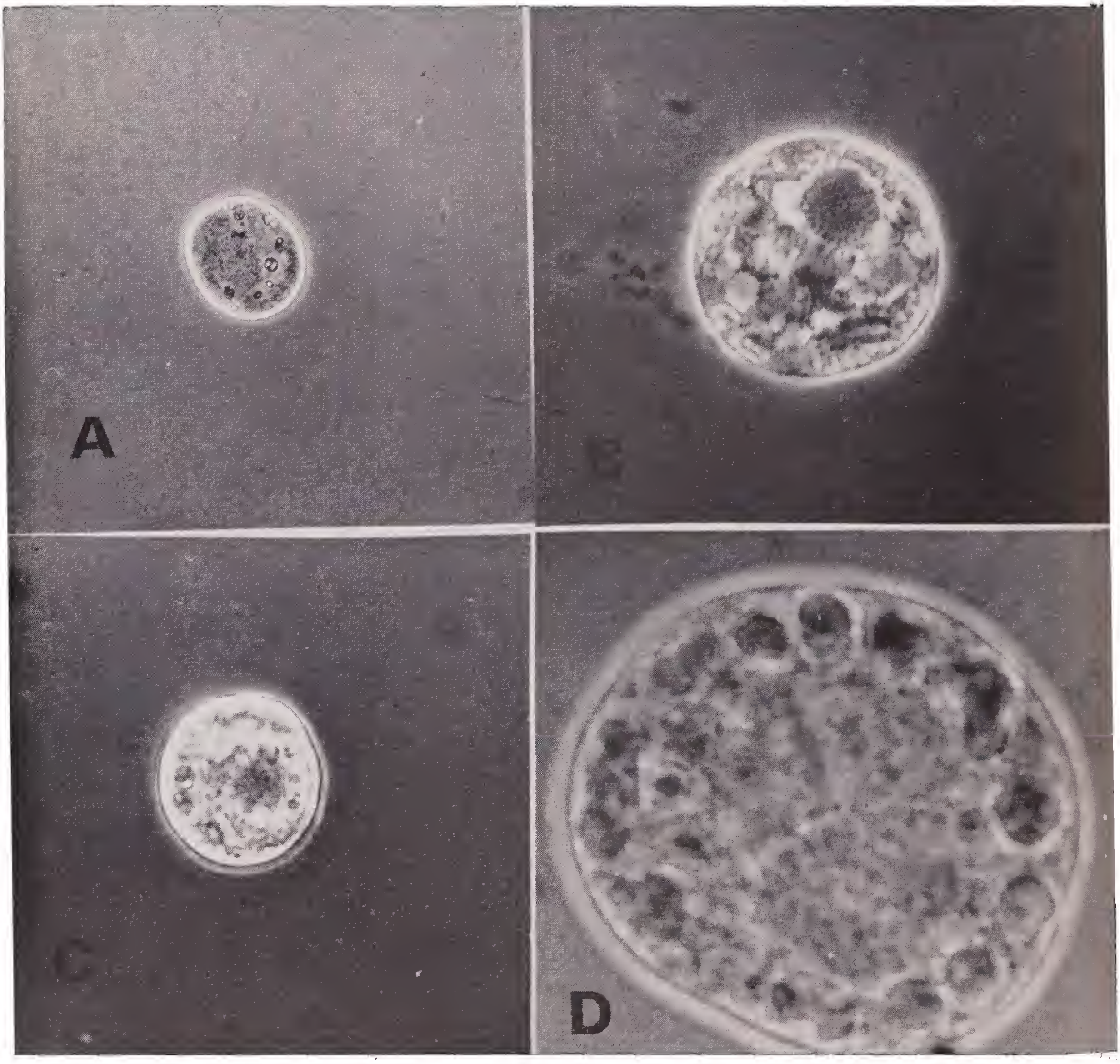

FIG. 5. Cells rounded by adverse conditions. Sloughed flagellum to left of cell in 10-B. (A—×240; B & C—×480; D—×1200).

The third cyst-like form occurred occasionally in cultures. It was similar to a normal cyst, but a closer examination revealed an enclosed membrane. Only one nucleus was observed in these cells and it was always within the inner membrane. Frequently the outer membrane became deformed after the inner membrane developed (Fig. 8).

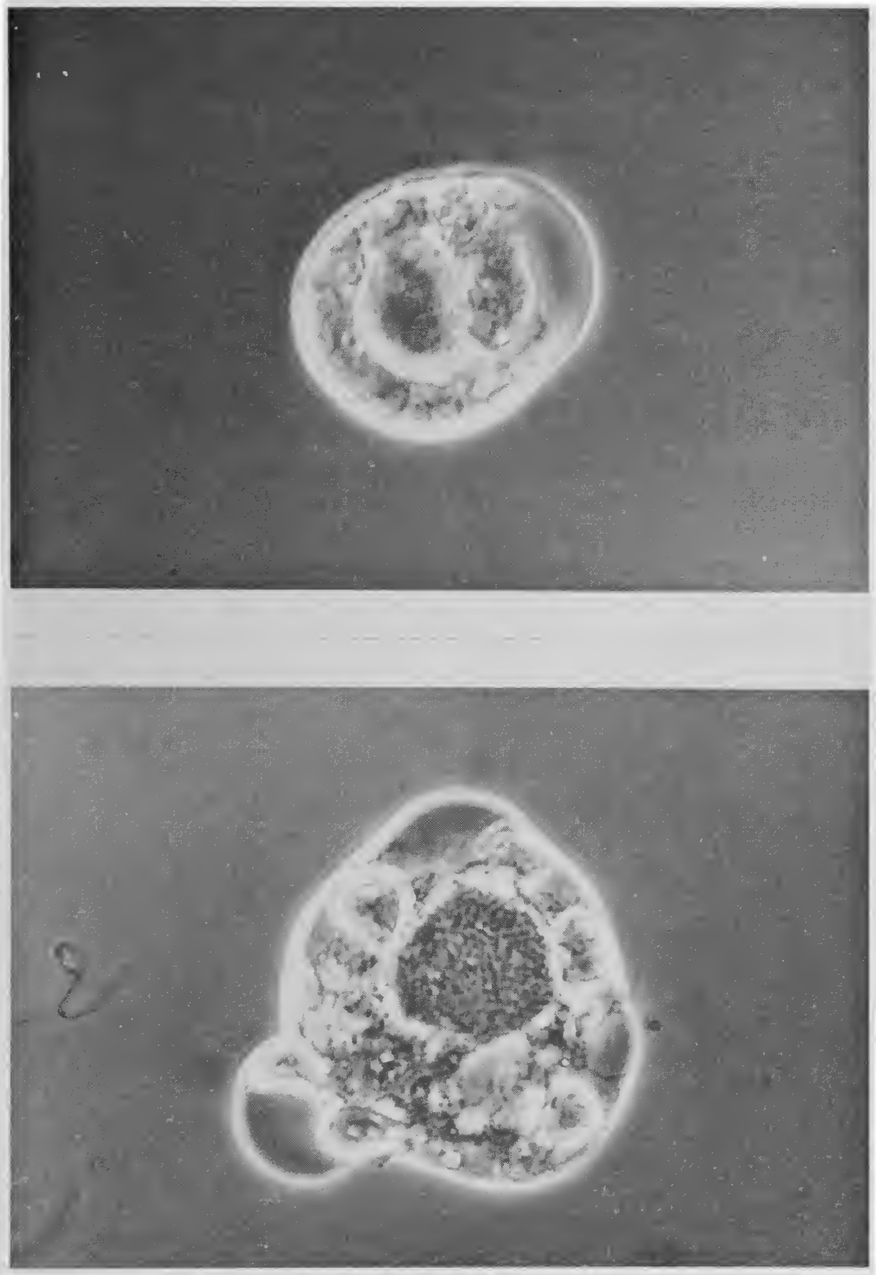

Fig. 6. Rounded cell with distended membrane (×480).

REPRODUCTION

The only method of reproduction that was observed through a complete cycle was binary fission. Certain observations suggested that *G. breve* may reproduce in other ways.

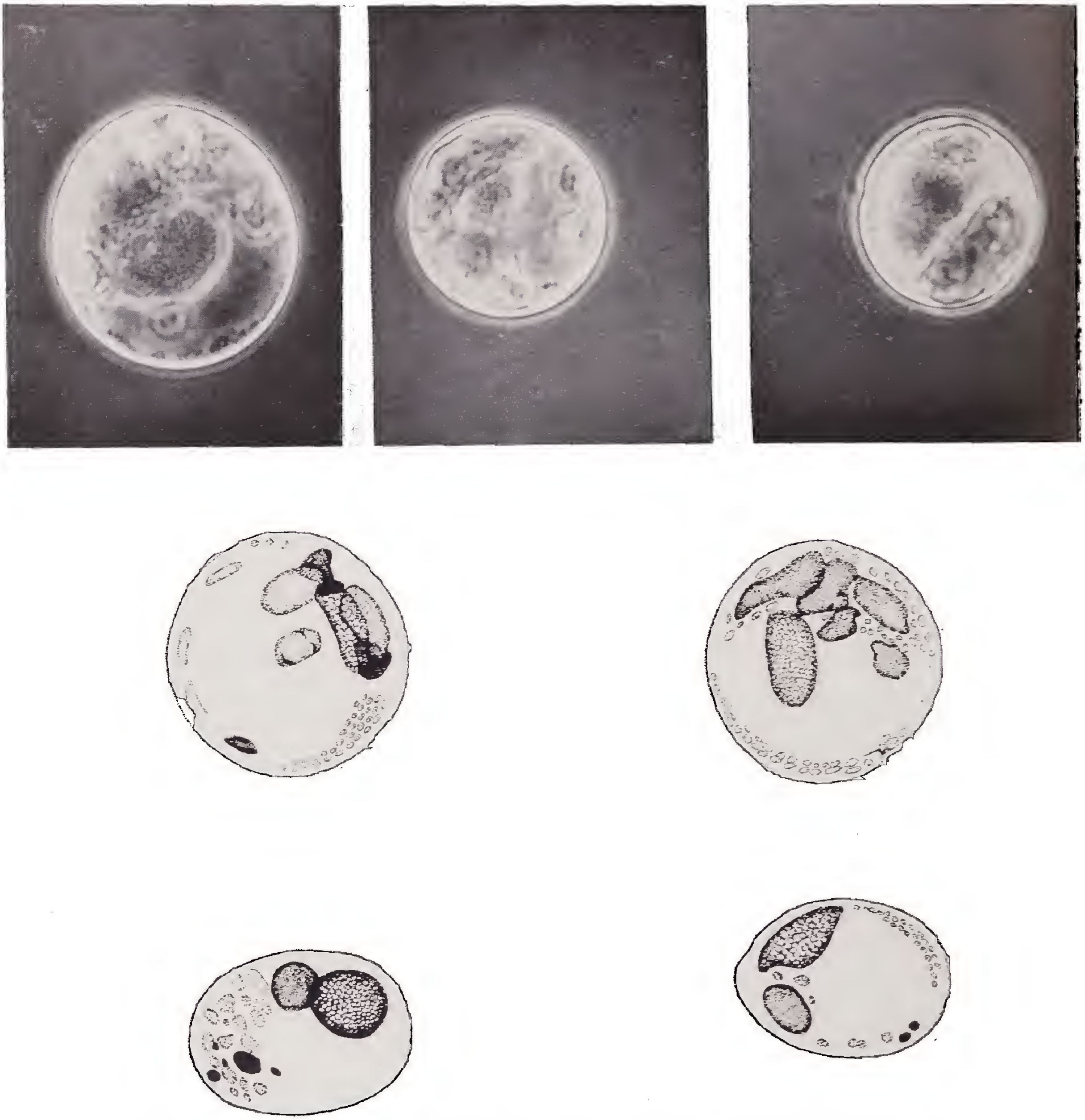

FIG. 7. Encysted *G. breve* cells. Sketches made from observation with bright field microscope (×480).

Cell division generally began after a cell had grown large. The first indication of cell division was the lateral expansion of the nucleus (Fig. 9). Before the daughter nuclei were formed, two posterior flagella could be seen. Although they were not easily observed, two transverse flagella were also present at this time. The latter were seen best by observing cells as they sloughed the flagella. Before any indication of cleavage of the cell, the daughter nuclei were well separated. Cleavage occurred along the sulcus. It usually began along the margin of the epicone followed by complete cleavage of the hypocone. This left the daughter cells joined at, or slightly above, the girdle. As cleavage proceeded the daughter cells turned relative to one another before they became completely separated (Fig. 10). As a result, they were usually oriented so that the girdle of one was almost at a 90° angle to the girdle of the other. Although cell division was apparently completed, daughter cells frequently remained together for periods longer than 2 hours.

In cell division, the period between the expansion of the nucleus and separation of daughter cells was between 13 and 24 hours. The average rate of cell division from the time of inoculation until maximum populations developed was calculated to be *ca* 0.4 division per day. The maximum rate during this period was *ca* 0.7 per day. Generally, the lag phase was 4 to 8 days, but was 30 days or more on some occasions. Lag phase duration and failure to grow was inversely related to the size of the inoculum.

In addition to cell division by the normal free-swimming cells, it is possible that cysts reproduced by cell division. Occasionally, cysts were observed which appeared to be dividing (Fig. 11). Kofoid and Swezy (1921) and Smith (1950) mention cell division by dinoflagellate cysts. In fact, Kofoid and Swezy considered that most dinoflagellate cell division took place while the organisms were in an encysted stage.

Kofoid and Swezy (1921) stated that a crescent-shaped (lunula) stage which undergoes multiple fission is to be expected for all species of *Gymnodinium*. Such forms were not observed in cultures of *G. breve*. Crescent-shaped cells from samples collected near natural *G. breve* populations that were transferred to

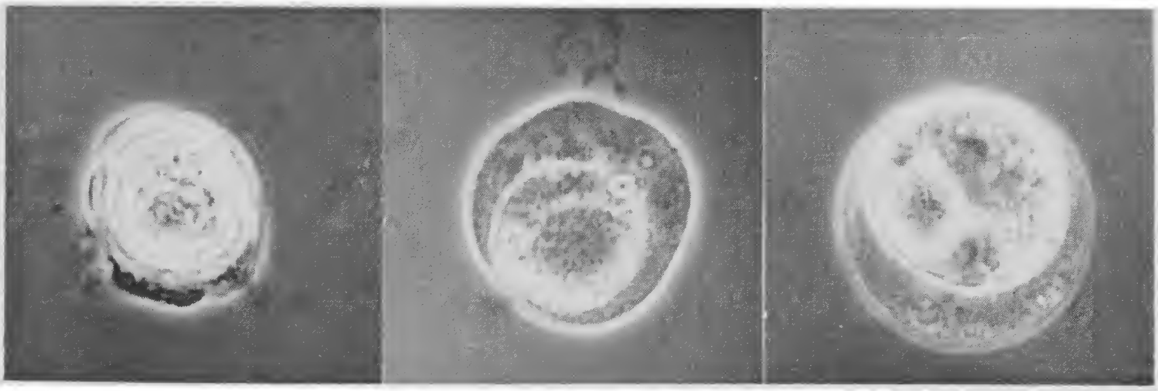

FIG. 8. Encysted stage with inner membrane (left and center) and deformed outer membrane (right) (×480).

fresh culture media produced small free-swimming *Gymnodinium* spp. that did not resemble *G. breve*.

On three occasions a condition occurred in *G. breve* cultures that was suggestive of conjugation. On these occasions, mature cells became joined in pairs by a tube (Fig. 12). Up to *ca* 40% of the population was paired-off in this manner. The position of the paired cells relative to one another and the position of the tube varied considerably from one pair to the next. No morphological changes were observed except the development of the tube and the presence of protoplasm

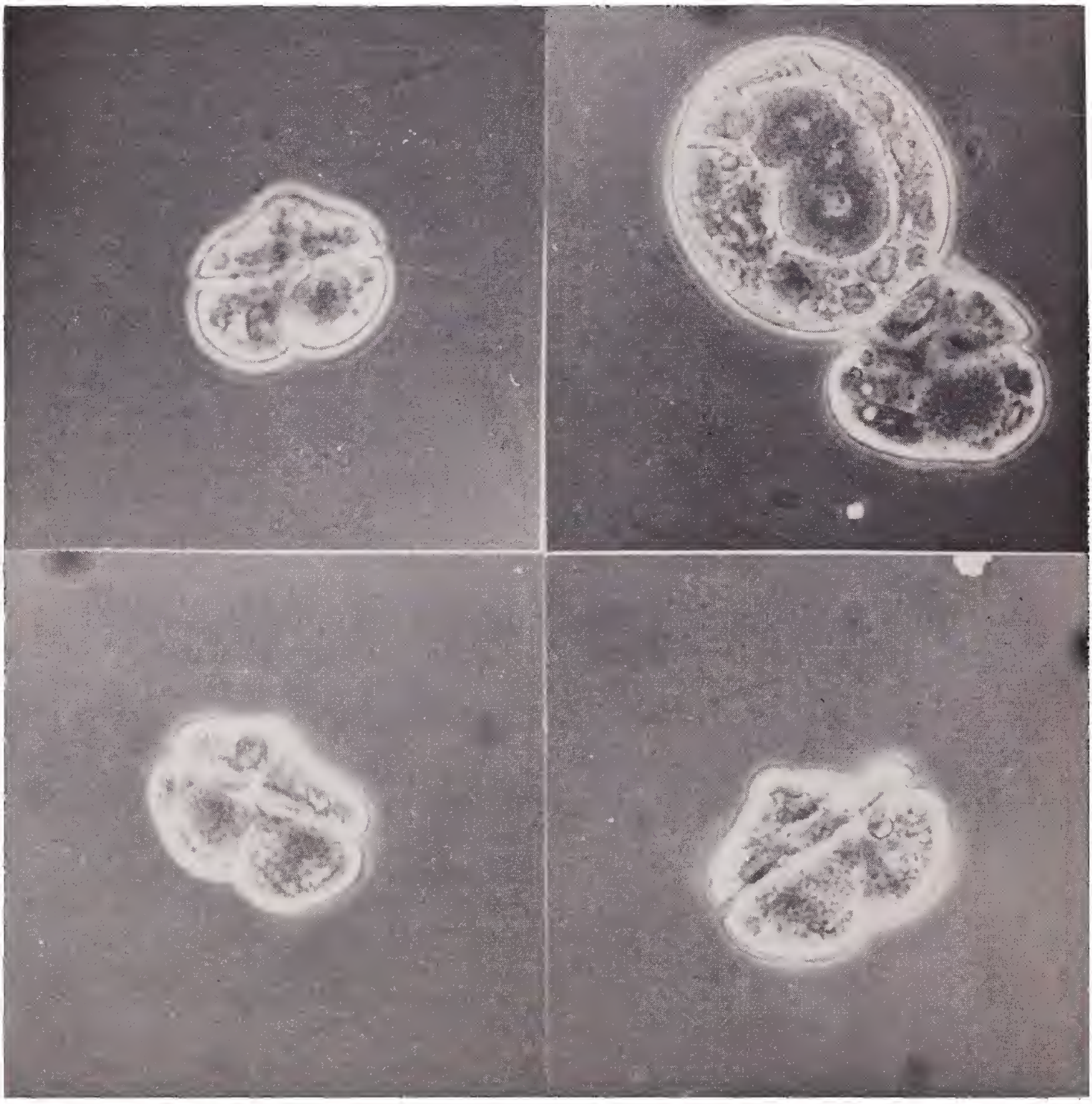

FIG. 9. Cells with laterally expanded nucleus (upper); daughter nuclei (lower left); and early cleavage along right epiconal extension of nucleus (lower right) (×480).

within it. Although joined cells were observed on only three occasions, no attempt was made to elicit this activity by manipulating cultures or culture conditions. The fact that a large portion of the culture population was paired in this manner; the apparent involvement of only mature cells; and the consistent presence of a connecting tube suggested that this may be an important stage in the life history of *G. breve*. Joined cells were observed only during periods when 10% to 50% of the culture was harvested between 3 and 7 times per week for a period of 3 or 4 months and the culture volume was maintained by adding fresh medium.

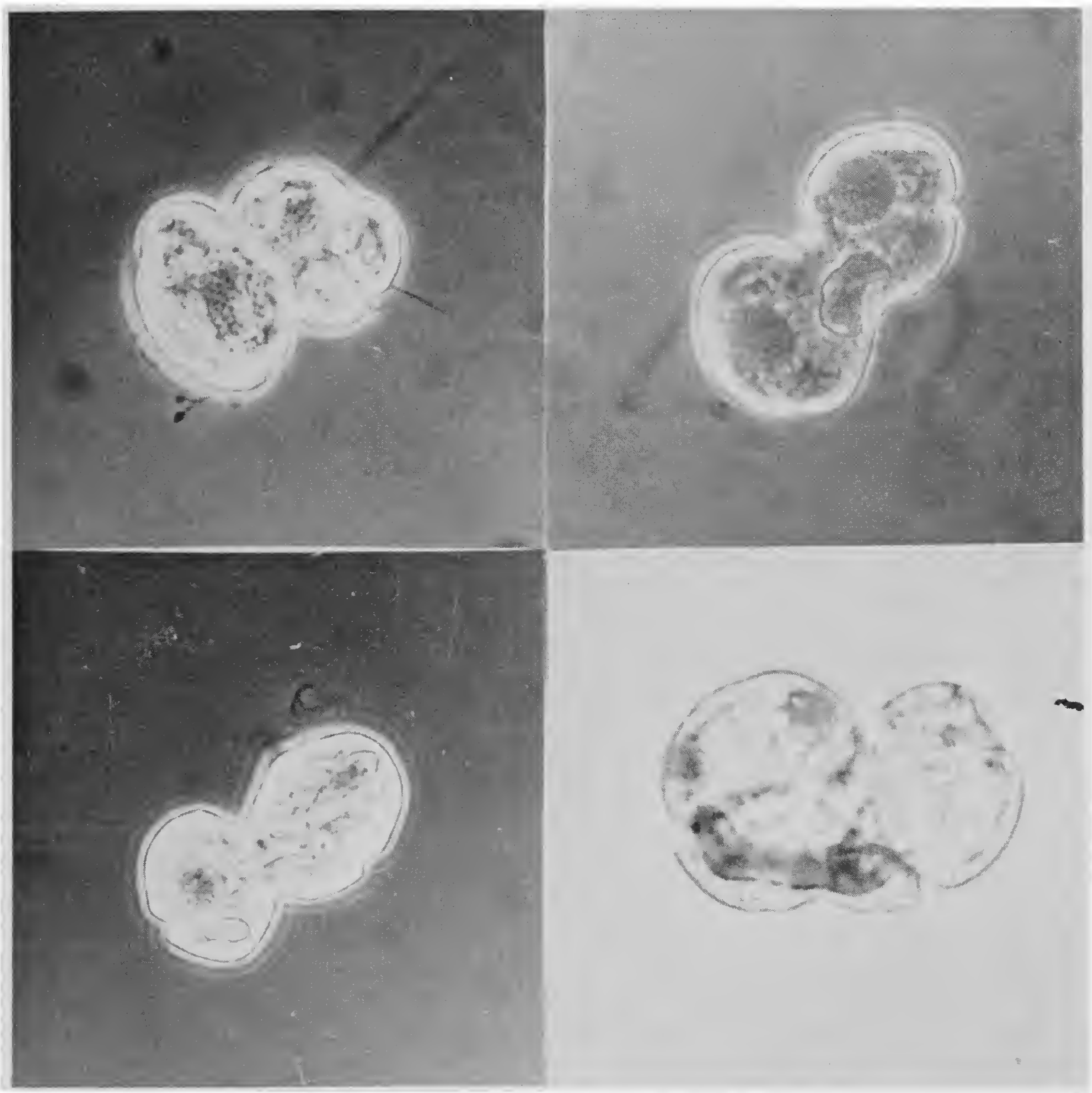

Fig. 10. Cell division. Mid and late stages of binary fission of *G. breve* (×480).

DISCUSSION

The pattern of growth in cultures over extended periods suggested that the life cycle of *G. breve* is a simple cycle of cell growth and cell division with the formation of cysts as the only intervening form. On the other hand, the occasional

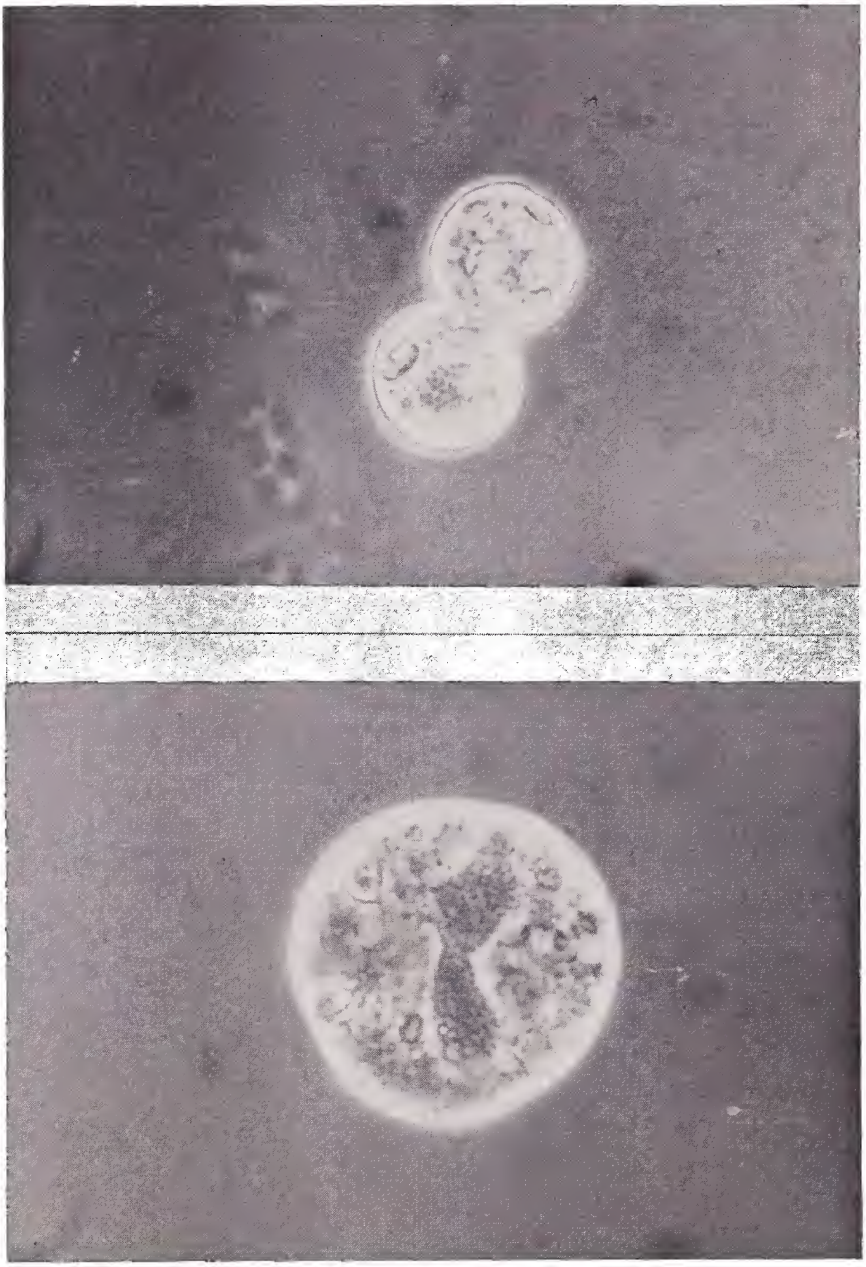

FIG. 11. Apparent cell division of encysted cell. Expansion of nucleus (upper); late stage of division (lower) (×480).

or rare occurrence of other forms may be indicative of a more complex life history.

Descriptions of sex and sexual phenomena of dinoflagellates are few. Zederbauer (1904) stated that *Ceratium hirudinella* produced a conjugate tube and formed a zygospore after fusion of the cell contents. The tube that joined pairs of *G. breve* may be a similar structure, however the fusion of the two individuals to form a zygote was not observed. According to Smith (1950), the fusion of free-swimming gametes of *Glenodinium lubiniensiforme* form a zygote which goes through a meiotic division before forming zoospores. The zygote is small and spherical with a smooth wall. More recently (von Stosch 1965), conditions were described which elicit a sexual process in *Ceratium cornutum* and a resulting formation of "microswarm" cells which in turn produce zygotes. The similarity of some of the isolated observations of *G. breve* forms to those reported for other dinoflagellates suggests that *G. breve* may possibly have a sexual cycle.

Most of the forms of *G. breve* observed in cultures, other than the normal free-swimming cells, were either non-motile or with reduced motility. Thus, these forms were found on the bottom of the culture vessel almost exclusively, suggesting that the place to search for them is in the littoral zone. Resting forms may be associated with plants or animals attached to the bottom. It is possible that resting stages of other organisms are present in the same location. If so, the various forms need to be cultured to be identified.

No description of *G. breve* can include all variations. Cultured forms exhibit polymorphism. Such variants may be caused by culture conditions or they may be stages in the life cycle; *e.g.*, the size, number and color of the chromatophores vary. Davis (1948) indicated that *G. breve* had several yellow-green chromatophores while Lackey and Hynes (1955) stated that the *ca* 22 discoid chromato-

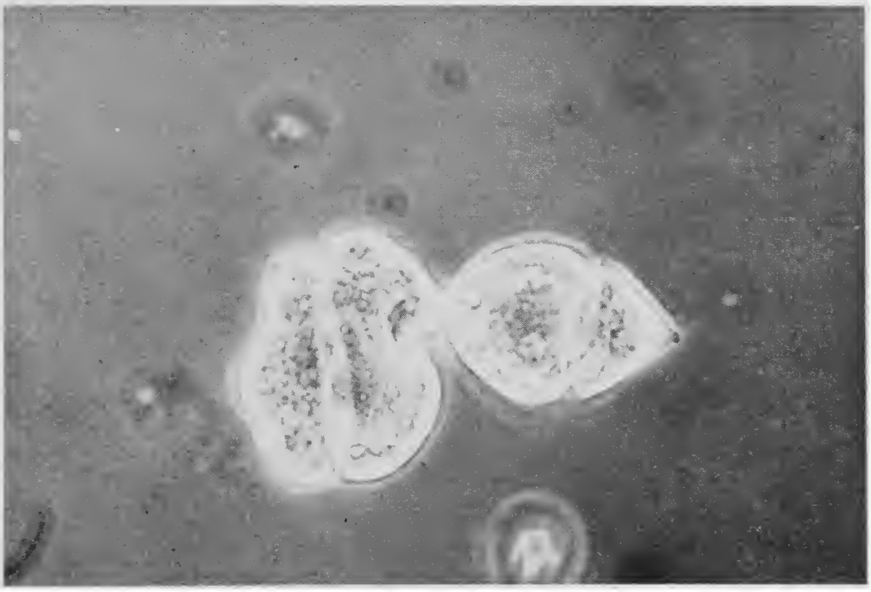

FIG. 12. Mature *G. breve* cells joined by a tube. Cell on left is in an almost lateral view. Cytoplasm in tube is contiguous with both cells (×480).

phores appear pale yellow-brown in transmitted light. My observations indicate there were from 1 to 20 chromatophores. The chromatophores were usually yellowish-green, however their color varied from greeenish-yellow to green. The nucleus also varied in size, shape and position in the cell. This feature may be associated with different stages in the life cycle (Dodge 1965). Thus, one may expect to find more morphological variations if there are other stages in the life cycle of *G. breve.*

ACKNOWLEDGMENTS

Part of the study was conducted at the Galveston Biological Laboratory, U.S. Bureau of Commercial Fisheries. The study was supported by the Florida Board of Conservation Marine Laboratory.

The author appreciates the critical review and suggestions of S. H. Hutner, L. Provasoli, R. M. Ingle, A. Collier and D. V. Aldrich.

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FATTY ACIDS IN RECENT SEDIMENT

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ABSTRACT

Fatty acids isolated from marine sediments of several environments were compared to acids isolated from marine plankton. An inventory of organic matter in sediments is contrasted with that in organisms. The geochemical significance of the branched-chain acids and the even-odd acid ratio is discussed. Several geologically early chemical transformations of fatty acids in sediments are pointed out.

INTRODUCTION

In recent years the fatty acids have been the subject of increasing study by organic geochemists. Fatty acids are major components of all living organisms. As such they may be an important source material for petroleum and perhaps for kerogen, the insoluble organic material found in sediments. Cooper (1962), Cooper and Bray (1963) and Lawlor and Robinson (1965) have investigated the fatty acids in ancient sediments and suggested that the normal paraffin found in sediments and petroleum may be derived from fatty acids. Fatty acids are among the most stable chemical species preserved in sediments. For this reason they are of interest as paleobiochemicals which may yield bits of information about very early life. Abelson *et al.* (1964) and Hoering and Abelson (1965) have isolated fatty acids from geologically very old rocks in which biochemical and isotopic evidence may be the only remaining evidence that these rocks were once associated with living communities.

In this study, fatty acids in very recent marine ecosystems have been measured and some of the geologically very early chemical transformations of fatty acids detected and evaluated. In Table 1 the fatty acid concentration in recent Gulf of Mexico sediment is compared to that of cartotenoids and sterols. Although acids are more abundant, they do not overshadow the others as one might expect a major constituent of organisms to do. It was felt that for recent sediment fatty acid geochemistry the best approach would be to try to understand the acids in the sediments in terms of the acids being produced by organisms.

EXPERIMENTAL

SAMPLES

All the recent sediment samples were frozen soon after the time of collection and kept frozen until used, except the British Honduras samples which were preserved in alcohol during transportation. The British Honduras samples were from a 3.6-meter core, consisting mostly of carbonate, taken from a typical back-reef environment. The core was donated by the Phillips Petroleum Co. The San Pedro Basin sample was a grab sample taken off California. It and the Green River Shale sample were donated by Dr. T. C. Hoering.

The other samples collected from the Texas and Mexican Gulf coast were grab samples and represent roughly the top five cm of sediment. Baffin Bay is a hypersaline arm of the Laguna Madre located about 30 miles (48 km) south of Corpus Christi, Texas. The ten Gulf of Mexico samples were taken off Port Aransas, Texas in from 20 to 200 feet (6–60 m) of water. Aransas Bay is a small bay five miles (8 km) north of Port Aransas and is isolated from the Gulf of Mexico by the same distance. The Mexican Laguna Madre sample was taken about 40 miles (64 km) south of the mouth of the Rio Grande river.

CHEMICAL PROCEDURE AND ANALYTICAL METHODS

The analytical procedure was designed to detect fatty acids containing 12 to 20 carbon atoms. This excludes only a small portion of the acids found in recent sediments. Care was taken to avoid excluding any fatty acid type. The urea adduction method was avoided because it excludes branched-chain acids. An attempt was made to minimize decomposition of the unsaturated acids. However, it is felt that in the plankton samples some of the polyunsaturated acids with more than twenty carbon atoms were lost.

TABLE 1

Organic chemicals from Gulf of Mexico sediments

Compound	Organic C percent	ug per g org. carbon	ug per g sediment	Reference
Carotenoids	0.6	300	1.8	Schwendinger & Erdman 1963
Sterols	0.6	107	0.7	Schwendinger & Erdman 1964
Fatty acids	0.75	1500	11	this work
Palmitic acid	0.75	320	2.4	this work

The frozen sediment sample was treated with dilute HCl to remove carbonate and washed with water to remove the inorganic salts. After filtration the moist mud was treated with methanol for 15 minutes while being stirred and subjected to ultrasonic vibrations. The methanol extract was recovered by filtration. The mud sample was again extracted using chloroform with stirring and ultrasonics. The recovered chloroform and methanol extracts were combined and the mixture almost completely dried with a rotating evaporator. This residue was taken up in chloroform and washed with 1N HCl to remove inorganic salts. The chloroform was removed by evaporation. After saponification of the residue with potassium hydroxide, non-saponifiable impurities were removed by extracting the alkaline solution with hexane. Finally the alkaline solution was acidified, and the fatty acids were extracted into hexane. Methyl esters were prepared by the BF_3 method (Metcalfe and Schmitz 1961). Plankton were treated the same way except the first treatment with HCl was skipped.

The methyl esters were identified and measured by gas chromatography on columns of diethylene glycol succinate (DEGS) and of Apiezon L. Standard fatty acid mixtures were used to calibrate a F & M model 400 instrument. On some samples unsaturated peaks were removed by bromination whereas the branched-chain peaks were not changed. Linear-log plots of the DEGS retention values yielded straight lines for the various series of acids and were a great help as confirming evidence (Woodford and van Gent 1960). Since the branched-chain acids had not been reported by most investigators to occur in sediment they were given extra attention. Confirming evidence was obtained from the infrared spectra of the combined iso and antesio C-15 peaks isolated from a large sediment sample. The peaks were collected at the exit port of an Aerograph 202 in a capillary tube and redissolved in carbon tetrachloride. Although the materials collected contained some normal C-15 acid the "isopropyl splitting" of the bond at 1380 cm^{-1} was apparent (O'Connor 1960). This is shown in Figure 1.

The organic carbon content of the sediments was determined by combustion with CuO on a vacuum line, freezing out the CO_2 and allowing it to expand against a calibrated manometer.

RESULTS AND DISCUSSION

The fatty acids from marine plankton was assumed to be the main source of the acids for the ecosystems studied. Larger organisms such as fish are present but their contribution is taken to be small. The fatty acid composition of the material collected in plankton tows using a 200 mesh net was determined. This material includes besides phytoplankton and zooplankton a large amount of non-living particulate organic matter. Since this material has already started to decompose it will qualitatively and quantitatively dilute the acids of living organisms. However, experience with pure cultures containing no such particulate material indicated that this material did not change the general ratio between acid types enough to affect our conclusions. In Table 2 the inventories of organic matter from a plankton tow and a bloom of the free-floating marine blue-green algae, *Trichodosemium erythraeum*, are contrasted with the inventories of two different marine sediments. The weight of the sample in each case was such that

100 mg of total organic carbon could be considered. For the organisms and the sediment more than 90% of the carbon is non-lipid. However, there is an important difference between these two non-lipid fractions. The non-lipid fraction of organisms can be broken down into simple biochemicals such as amino acids and sugars while the non-lipid fraction of the sediment is mostly kerogen, and thus can not be broken down into simple biochemicals (Forsman and Hunt 1958). The importance of this difference is that sedimentary organic matter is not simply dead organisms. The sediments contain about the same fraction of lipid carbon

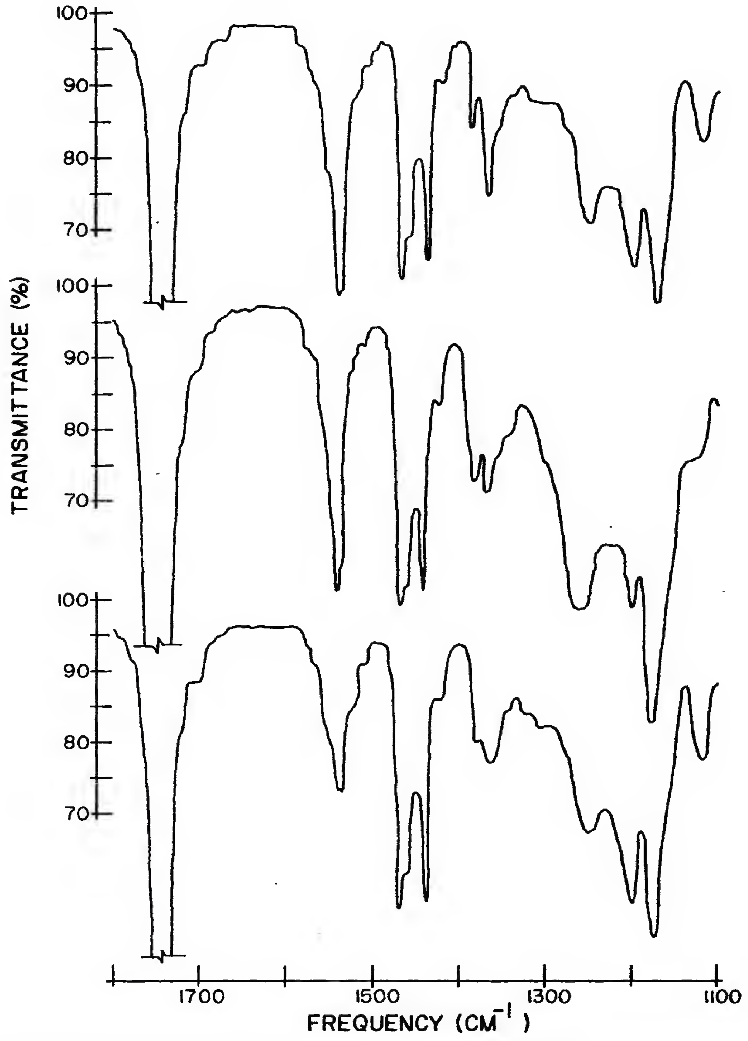

FIG. 1. Infrared Spectra of Fatty Acids (esters of). (top) methyl isotetradecanoate (i-C14), standard. (middle) methyl isopentadecanoate (i-C15), sample peak collected from GLC. (bottom) methyl pentadecanoate (C15:O), standard. *Note*: All of the above species were in solution in carbon tetrachloride. The substance whose spectra appears in the middle figure was not pure but contained a-C15 as well. Iso acids can be distinguished from their corresponding anteiso and normal forms in that they show a doublet in the range from 1360 and 1380 cm^{-1}.

TABLE 2

A comparison of organism and sedimentary carbon

	Trichodesmium erythraeum	Plankton Tow	Baffin Bay	Gulf Mexico
Weight g	1	1	6.5	16
Total org.-C	100 mg	100	100	100
Non-lipid-C	93.1	90.7	93.8	96.5
Lipid-C	6.9	9.3	6.3	3.5
Fatty acid-C	1.2	2.4	0.4	0.07
16:0	0.14	0.6	0.1	0.02
18:3	0.08	0.1	n.d.	n.d.

Sample weights adjusted to give 100 mg of Total organic carbon. All weights in mg. n.d. means not detected. 16:0 refers to the straight chain fatty acid with 16 carbon atoms and no double bonds; 18:3 refers to the straight chain fatty acid with 18 carbon atoms and 3 double bonds.

as the organisms. The relative chemical composition of the organism and sediment lipid is not known. However these fatty acid data show: (1) the sediments are depleted in fatty acid with respect to organisms; and (2) not much of the sediment lipid carbon is fatty acid, even in the case of the Baffin Bay sediment which is one of the fatty acid richer sediments studied. Complete chemical characterization of the sediment lipid fraction remains an unsolved proglem for organic geochemists. Palmitic acid is almost always the most abundant acid in sediments. The polyunsaturated acids are not found in the sediments although they are a major acid in most organisms. Parker and Leo (1965) found that while living blue-green algal mats contain substantial amounts of unsaturated acids the underlying old mats become progressively depleted in unsaturated molecules. This early disappearance of unsaturated acids may be due to their being converted into a kerogen type material or into other organic molecules.

The model of plankton supplying fatty acids to the sediments seemed simple but it was necessary to modify it. Unidentified peaks that appeared regularly in gas chromatograms of sediments, but which were absent or minor in organisms, proved to be branched chain fatty acids (Leo and Parker 1966). These com-

TABLE 3

A comparison of the fatty acids from Aransas Bay plankton to the fatty acids from recent sediment

Sample	$\frac{14:0}{i14:0}$	$\frac{15:0}{i+a\ 15:0}$	$\frac{16:0}{i16:0}$	$\frac{16:0 + 18:0}{15:0 + 17:0}$
Plankton	56.	6.	180.	8.
Aransas Bay (9)	4.7	1.1	11.	2.3
Gulf of Mexico (10)	7.4	0.85	9.	2.4
Laguna Madre, Mexico	3.1	2.9	10.	5.4
Baffin Bay (9)	2.7	0.4	8.5	5.8
British Honduras (7)	4.0	1.2	14.	6.1
San Pedro	6.0	0.88	21.	3.5
Green River Sh.	5.7	4.0	24.	5.9

Numbers in parentheses indicate number of samples averaged.

pounds, iso and anteiso acids, had not previously been reported for sediments. The ratios of straight chain to branched chain acids for plankton and sediments are shown in Table 3. The iso and anteiso fatty acids are greatly enriched in the sediments as compared to plankton. While higher marine organisms contain little branched-chain fatty acid, bacterial lipids are noted for being rich in branched-chain acids (Shorland 1962). Kaneda (1963) has shown that the major fatty acids of *Bacillus subtilis* are the iso and anteiso acids. Two of the five ciliated protoza studied by Erwin and Bloch (1963) contained significant amounts of iso acids. Both bacteria and protozoa live in the upper sediment, deriving food from organic detritus and perhaps producing enough branched-chain acids to account for the enrichment observed. The branched-chain acids are present in all the sediment samples studied (Table 3). Parker, Van Baalan and Mauer (1967) isolated four species of bacteria from Baffin Bay sediment. They found that branched-chain acids were the most abundant acid for two of the species. Thus, a large part of the branched-chain acids is probably derived from bacteria. Peterson (1967) has recently isolated these same branched chain acids from recent sediment. He also concludes that bacteria are the most likely source.

It is well established that odd carbon number saturated fatty acids are present in both recent and ancient sediments (Williams 1965). Cooper and Bray (1963) and Kvenvolden (1966) have suggested that the odd acids are derived from even acids, basing their conclusions on a comparison of the distribution of straight-chain paraffins and fatty acids of recent sediments and ancient sediments. Jurg and Eisma (1964) have shown experimentally that hydrocarbons can be derived from fatty acids under conditions not geologically unreal. The ratios of the major even and odd carbon number fatty acids shown in Table 3 indicate that in Aransas Bay the sediment is enriched in odd acids over the plankton. This is so, although the ratio of eight shown for these plankton is much lower than the same ratio for several pure cultures of blue-green algae studied (Parker, Van Baalen and Mauer 1967). The source of the odd carbon acids which cause the enrichment is not known.

The variation in the ratios of Table 3 for samples from different environments is significant. There is usually less variation in these ratios for samples from the same environment. Peterson (1967), in a detailed study of fatty acids in sediments along the Washington coast, found strong evidence that different environments are characterized by different fatty acid ratios.

A detailed study of the branched-chain acids in ancient sediments would bear on the origin of odd carbon acids in ancient sediments. The 15 carbon branched-chain acids are the most abundant branched-chain acids in recent sediments (Table 4). Therefore, if chain shortening has taken place in ancient sediments, as Cooper (1962) suggests, then the 14 carbon branched-chain acids should be enriched with respect to the 15 carbon ones.

Average results of complete fatty acid analyses of recent marine sediments from three different environments are given in Table 4. The data are expressed on the basis of sediment weight but the percent organic carbon are given. These numbers represent minimum concentrations because there is no way to know what fraction of acid is extracted from sediment. Although the error may be high it is important to report concentrations so that data may be evaluated. The samples reported in this study are representative of several hundred sediments from this area which have been studied.

In summary, several points bear repeating. Much of the geochemical modification of fatty acids takes place early. The unsaturated acids so abundant in marine organisms rapidly fall to low values in sediment; the fatty acid content of recent sediment falls to a value similar to that found in ancient sediment; and enrichment of at least one species, the branched-chain acids, takes place. Further study of fatty acids from different recent environments would add to our understanding of these substances. Information on fresh water systems might be especially useful for recognition of such environments in the geological record.

ACKNOWLEDGMENTS

Technical assistance was supplied by Jean Copeland. Supported by NSF grants GP-2515 and GA-299.

TABLE 4

Concentration of fatty acids in recent sediments

	microgram acid per gram sediment		
Acid	Aransas Bay (9)	Baffiin Bay (9)	Gulf of Mexico (10)
12:0	0.34	0.73	0.19
13:0	0.17	0.21	0.05
i14:0	0.32	0.97	0.13
14:0	1.5	2.6	0.7
14:1	tr	0.5	0.07
a+i15:0	2.5	3.1	0.97
15:0	2.7	1.3	0.82
15:1	0.59	tr	0.2
i16:0	0.68	1.3	0.26
16:0	7.4	11.	2.4
16:1	4.5	5.5	1.7
i17:0	0.96	1.1	0.67
17:0	1.4	1.1	0.35
17:1	2.6	0.8	0.42
i18:0	0.23	0.84	0.2
18:0	1.9	3.0	0.45
18:1	4.5	7.5	1.1
F.A.	32.0	42.0	11.0
% org. C	0.6	1.6	0.5

Numbers in parentheses indicate number of samples averaged.

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METABOLIC RESPONSES OF SOME ESTUARINE ORGANISMS TO AN INDUSTRIAL EFFLUENT[1]

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ABSTRACT

The oxygen consumption rates of several species of estuarine organisms were measured in various concentrations of a petrochemical company effluent. Test solutions used were of concentrations less than the TL_m value, as determined by the toxicity bioassay method. It was learned that both short-term and chronic exposure exert stress conditions causing organisms to experience changes in metabolic requirements. A typical response was a decreased metabolic rate in low concentrations and an increased rate upon prolonged exposure or in concentrations approaching the TL_m value. However, responses may vary with the type effluent used, length of exposure and with the particular species under study.

These data were interpreted with respect to the ecological requirements of individual organisms and entire populations. It is apparent that populations may experience gross deleterious effects, without apparent "fish kills," under what may normally be interpreted to be "safe" pollution levels.

INTRODUCTION

Concern over disposal of wastes into freshwater supplies results primarily from the potential threat this practice poses to the quality of water intended for human use. The recent and increasing industrialization of coastal areas, however, has extended the threat of pollution to estuarine waters. Because of the unique nature of saline water, most such areas are of reduced value for domestic or industrial use *per se.* The economic value of such areas lies in their potential for commercial and recreational use, depending upon their suitability for habitation by fish, crabs, shrimp, shellfish and for other natural resources. Therefore, the major concern with disposal of wastes into such areas should, even more than for water supplies, be directed at the effects of artificial eutrophication and toxicants.

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[1] Modified from a thesis presented by the first author to the Graduate School, The University of Texas, in partial fulfillment of the requirements for the degree of Master of Arts, January, 1967.

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It is essential that different and more encompassing studies be designed to assess the effects of pollution of marine and estuarine systems. The standard procedures used in freshwater studies are a beginning, but by themselves, are inadequate even in freshwater. Short-term bioassay methods, such as those described by Douderoff *et al.* (1951) and the American Public Health Association (1965) for evaluating acute toxicity of effluents to fish populations, have little meaning when one considers the implications of chronic exposure to "sub-lethal" concentrations of toxicants and the possible resulting inhibition of normal physiological processes. Usually, these tests deal with concentrations rarely reached in a natural system. Long-term bioassays, such as those conducted by Graham (1963), are more realistic and further emphasize the inadequacies of the former method. Median tolerance limits as determined by extended period tests are much lower, and even the surviving fish are found to be severely emaciated. However, these methods are still lacking in comprehensiveness.

Numerous investigations of the acute effects of various toxic compounds on freshwater fish have been made. Reviews of these studies are given by Douderoff and Katz (1950; 1953), Beak (1958) and more recently by Jones (1962). However, extension of these studies to estuarine and marine organisms has been very limited. The chronic exposure of *Leiostomus xanthurus* to trace amounts of toxaphene have been investigated (Lowe 1964). Alderdice and Brett (1957) and Haydu *et al.* (1952) studied some effects of papermill effluent on young Pacific salmon. Young salmon also were used as test organisms by Williams *et al.* (1953) in toxicity studies with sulphite waste liquor.

Brett (1958) suggested the use of physiological criteria for assessing the presence of an indiscriminate or population stress such as chronic and/or sub-lethal toxicity. Because metabolism is an integrative characteristic, any stress condition placed upon an organism should be reflected by changes in metabolic rates. When critically evaluated, a metabolic indicator such as oxygen consumption rates may be useful in assessing the physiological state of an organism. In the case of fishes, many data on oxygen consumption rates are available to facilitate ecological interpretation.

Investigations of respiration of fishes exposed to pollutants have been primarily concerned with the effect of reduced oxygen tension. Whitworth and Irwin (1961) discussed the problems of reduced oxygen tension and presented an extensive bibliography on the subject. Because most industrial wastes have a high content of readily oxidizable materials, waters receiving them are rapidly depleted of oxygen. It is known that this stress condition is reflected by the respiratory rate of fish (Fry 1957; Beamish 1964a; 1964b) and is especially critical during certain life stages (Alderdice *et al.* 1958). The literature, however, reports few investigations of changes in oxygen consumption rates as a direct result of the presence of a toxicant. Jones (1962) reported changes in opercular movement rates as a result of exposure to metallic ions that interfere with oxygen removal by the gills. Smith *et al.* (1965) reported increased hematocrits for fish subjected to suspended papermill fiber, which suggests respiratory difficulty since the same response was noted when fish were under decreased oxygen levels. MacLeod and Smith (1966) found that active metabolism of *Pimphales promelas* was reduced in water containing suspensions of wood fiber. Similar work was done by Smith *et al.* (1966) who found that walleye fingerlings exposed for 190 days to suspended groundwood fiber had routine metabolic rates 18% higher and active metabolic rates 15% lower.

Respiratory distress in some marine organisms subjected to polluting substances has been noted. Hood (1960) detected metabolic changes in *Artemia salina*, the brine shrimp, following exposure to various wastes and to phenol. Copeland and Wohlschlag (1967) examined routine metabolic changes in pinfish exposed to polluted water.

Other criteria which have been used to assess the physiological state of fish in polluted systems include swimming speed (MacLeod and Smith 1966), opercular movement rate (Jones 1962), time for exhaustion to occur at a given swimming speed and the percent of fish able to swim at a given speed for a specified length of time or distance (MacLeod and Smith 1966). These methods are merely tests for endurance and do not allow for ecological interpretation as do changes in oxygen consumption rates.

It was the purpose of this study, using oxygen consumption rate as an indicator, to determine changes in the physiological state of some estuarine fishes and invertebrates subjected to sub-lethal and chronic toxicity from an industrial waste.

METHODS

Animals collected in the Port Aransas area and used in the oxygen consumption experiments consisted of *Cyprinodon variegatus* Lacépède, the variegated cyprinodon; *Lagodon rhomboides* (Linnaeus), the common pinfish; *Micropogon undulatus* (Linnaeus), the Atlantic croaker; *Penaeus duorarum* Burkenroad, the pink shrimp; and *P. aztecus* Ives, the brown shrimp. These animals were chosen because of their relative abundance, ease of collection, ecological significance and adaptability to the laboratory.

During the experiments, the animals were held in the laboratory in a covered wooden box (131 cm by 131 cm by 46 cm), constructed of ⅝-in. (1.6 cm) plywood and insulated with thick sheets of styrofoam. The temperature of this system was controlled by a circulating waterbath (30-liter capacity) fitted to 36.6 m of 1.6-cm plastic tubing coiled about the walls of the tank. Water was regulated to a chosen temperature and circulated through the coils at a rate of approximately four liters per minute. Water temperature in the tank changed relatively slowly and could be controlled to within 0.5 C over a range between 10 C and 33 C.

Darkened 4-liter bottles were used as metabolism chambers. The bottles were sealed with two-holed No. 14 rubber stoppers fitted with glass and tygon tubing to facilitate the removal of a volume of water for analysis. The tube draining to the sample bottle removed water from the bottom of the metabolism chamber. An equal refill-volume entered the J-shaped second tube, remaining near the top of the bottle. A number of trials, using a dye in the refill-volume, satisfactorily showed that no mixing with the incoming water occurred during the time necessary for siphoning a sample. Flow rates from the discharge tube were regulated by means of a pinchcock. The refill tubing was joined by an interchangeable ground-glass joint to a tubing from the bottom of a 500-ml graduated cylinder which served as a reservoir for the refill volume. The graduated cylinder was about one meter above the level of the metabolism chamber, which allowed a more rapid filling of the sample bottles. The refill-volume for all samples was maintained constant, which facilitated computations of changes in dissolved oxygen concentration due to the water exchange. Such a system for removal of a sample volume eliminated difficulties due to diffusion of oxygen across the water surface. Oxygen concentration of the refill water was monitored to assure saturation.

Organisms used in each series of metabolic measurements were placed in the temperature-controlled water, which was slowly raised or lowered to the desired temperature. Constant aeration assured that oxygen concentration in the water in the holding tank remained constant. An overnight acclimation period was allowed for each group of animals. When a dilution of the effluent was used, the desired concentration was mixed in the holding tank and organisms were given three to four hours of exposure to the effluent dilution before any measurements were made. Water from the temperature-control tank was used to fill the metabolism chambers. One animal was placed in each chamber, with minimum disturbance during removal from the tank. Twenty to thirty minutes acclimation were allowed before measurements were begun. Preliminary tests indicated that this was sufficient time for recovery form the initial excitation of being placed in the chamber. Salinities were kept between 29 and 34 ppt. During an individual experiment, however, variation was usually less than one ppt.

The chambers were submerged in the tank while each experiment was in progress. The above practices allowed for maintenance of constant temperature, salinity and for consistent handling of each animal.

Each animal was kept in its container from one to four hours, depending upon the rate of oxygen depletion. Between 10 and 25 animals were used in each group with two to four measurements for each animal. Each value in the final set of data was based on 20 to 60 measurements. Experiments were terminated before the oxygen reached about 50% saturation. Oxygen levels below this value cause respiratory dependence in many species (Fry 1957). Each experiment was conducted at approximately the same time of day to avoid any diurnal variation in metabolism (Fry 1957).

One experiment was conducted to detect any metabolic changes in *Cyprinodon variegatus* surviving under chronic toxicity of a 5% concentration of the petrochemical waste for a one-week duration. The fish were placed in the temperature-control tank immediately after capture where they were brought to the desired temperature. The effluent solution was renewed every second day because of decreasing toxicity. Some deaths occurred to fish in the holding tank, but those that appeared to be dying were excluded from experiments. A control experiment was conducted using a newly captured group of fish, of which none died, and their metabolic response was measured daily in a similar manner.

Oxygen consumption rates for the test organisms were calculated from changes in total oxygen content of the metabolism chambers per unit time. Allowances were made in the calculations for removal of oxygen with the sample volume and addition of oxygen with the refill-volume. Since the refill-volume remained near oxygen saturation, corrections were made for a gain in dissolved oxygen content of the chamber when successive measurements were conducted. No corrections for displacement of water by the organism in the chamber were necessary because the error introduced was negligible (0.7% maximum volume error, assuming specific gravity of unity). Each sample was flushed with the same volume in order to have consistency in sampling technique. Analysis for dissolved oxygen was made by the unmodified Winkler method (A. P. H. A. 1962). After completion of experiments the specimens were killed, blotted dry and weighed to the nearest 0.01 gm. For each group of measurements a control or blank run (containing no animal) was run in order to determine any oxygen depletion from bacterial respiration or chemical oxidation of effluent components.

Final data from computations were expressed as an average oxygen consumption rate for the period of measurement, in mg oxygen hr^{-1}. The control value for the chamber containing no animal was subtracted and the resulting value converted to oxygen hr^{-1} gm body $weight^{-1}$. Computations were made according to the following formula:

$$\text{mg } 0_2\text{hr}^{-1}\text{gm}^{-1} = \frac{A - B}{\text{wt. of fish (gms)}};$$

where, $A = [V_e\,(y\text{-}x) + V_c\,(x\text{-}z)]\,T^{-1}$,

B = same calculation without organism (blank),

x = oxygen concentration (mg 1^{-1}) in metabolism chamber at beginning of experiment,

y = oxygen concentration (mg 1^{-1}) in graduated cylinder,

z = oxygen concentration (mg 1^{-1}) in metabolism chamber at end of experiment,

V_e = exchange volume,

V_c = volume of metabolism chamber, and

T = time of experiment in hours.

Effluent from a petrochemical plant near Corpus Christi, Texas was used in the experiments conducted. The plant uses crude oil for production of gasolines, aviation fuel, organic solvents, and numerous other materials. The effluent is typical (high organic content) of the enormous quantities being released into Texas coastal waters by many similar installations in the area.

Toxic components of the effluent included chlorides, sulfates, sulfides, phenols, oil and suspended solids. Discussion of specific toxicity of all these components to various species of fishes are referred to in the bibliographies of Douderoff and Katz (1950, 1953) and by Jones (1962). Chemical composition of the industrial waste remained relatively consistent throughout the period of study, as compared to the high variability seen in most industrial wastes (Dorris *et al.* 1961). Characteristics of the effluent for the period January through September 1966 are presented in Table 1. Oxygen consumption experiments were conducted between March and September 1966. The 48-hour median tolerance limit (TL_m) for *Lagodon rhomboides* ranged from 8.8–11.8% effluent in clean bay water. Therefore, overall toxicity for the nine-month period shown remained practically within the limits of accuracy of the method.

Forty-eight or 96-hour TL_m concentrations for each type of animal used were determined by the method of Douderoff *et al.* (1951). Series of experiments were conducted with effluent concentrations between zero and the TL_m (48 hour) value so that possible metabolic changes at sub-lethal or chronically toxic concentrations could be determined. On occasions when test specimens died during or shortly after the experiment, data were discarded to avoid inclusion of "abnormal" measurements. Again, this would seem to bias the data, but it was intended to show metabolic changes only in those fish appearing to survive.

RESULTS AND DISCUSSION

Brett (1958) presented a working definition of stress as "a state produced by any enviromental factor which extends the normal adaptive responses of an animal, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced." He further specified that so long as enviromental changes do not extend beyond the range of an organism's adaptive abilities a stress condition does not exist. Such changes are considered to be only extremes of normal environmental conditions.

There are two main categories of stresses. One is a discriminate stress, which is applied to individual organisms without effect upon the remainder of the population. Stresses of this nature include parasitism, predation, trauma, etc. The other is the indiscriminate stress to which an entire population is exposed. However, individual responses to indiscriminate stress may differ, resulting in fractional mortality in most cases. Indiscriminate stresses include almost all physical and chemical changes in the environment.

TABLE 1

Characteristics of the industrial effluent used for oxygen consumption experiments

	Concentration (ppm)										
Date	"P" Alkalinity	Total Alkalinity	Total solids	Susp. solids	Cl^-	$SO_4^=$	Oil	$S^=$	Phenol	pH	% 48-hr TL_m
18 Jan. 1966	0	321	2461	196	1220	561	9.8	55	42	6.5	9.2
17 Feb. 1966	0	342	2396	221	1346	486	12.6	40	36	6.8	10.8
22 March 1966	0	280	2291	184	1122	430	24.2	35	28	6.9	8.9
12 April 1966	0	275	2150	150	1195	520	18.4	50	41	7.0	9.1
9 May 1966	0	280	2197	155	1220	397	5.4	35	26	6.6	9.2
21 June 1966	0	247	1590	195	970	485	35.0	50	45	7.0	9.8
6 July 1966	0	235	2495	126	1650	420	2.8	12	32	6.8	9.5
4 Aug. 1966	0	220	3295	250	1427	485	0.0	16	27	6.7	11.8
6 Sept. 1966	0	225	2450	260	1050	436	35.0	35	40	6.9	8.8

By Brett's definition, exposure of a population of aquatic organisms to the toxic components of an industrial waste may constitute an environmental stress. Furthermore, it is one of the indiscriminate variety, working upon the entire population concerned. Data from experiments reported here indicate that aquatic organisms respond to stresses of various natures as hypothesized by Brett.

The term "stress" as used in this discussion will be in context of that described above. Those instances where populations are spoken of as "adapting" to stress conditions (Nicholson 1954) will have no bearing here as their scope is excluded by definition. However, this does not exclude the possibility of a trend toward a compensatory reaction to a stress. The two main categories of stresses can be further divided on the basis of how organisms are affected.

1. An inhibiting stress acts to reduce the organism's ability to carry out normal functions. Any condition serving as a metabolic depressor, or one causing enzymatic imbalance, falls into this category.
2. Any condition bringing about an increase in an organism's metabolic cost acts as a loading stress. This includes high temperature, abnormal osmotic conditions, excitation, or any other energy-demanding or fatigue-causing stress.
3. A limiting stress resulting from an insufficient supply of metabolites brings about a decrease in activity.
4. A lethal stress results in death of the organism, and can be the final phase of almost any of the other types.

The first two of these are of primary concern in this discussion.

In each metabolism chamber, the fish was free to swim at random (spontaneous activity); however, no measurement of activity was made. Therefore, a portion of the oxygen was consumed for basic maintenance cost (standard metabolism) and the remainder due to "spontaneous activity" under chamber conditions (Fry 1957). Standard metabolic rate is normally defined as that measured "at rest" in the post-absorptive stage. Routine metabolism reflects minor changes in physical activity due to spontaneous movements and is essentially what is being measured in these experiments. Active metabolism is total respiration when the animal is actively swimming. In the following discussion, "normal" measurements refer to routine metabolism of the control animals, although this rate probably approaches a realistic standard rate.

METABOLIC RESPONSES BY FISH

Cyprinodon variegatus

The oxygen consumption rates of *Cyprinodon* at 20 C were measured in eight concentrations of the petrochemical effluent (*i.e.*, eight different stress conditions) having a 48-hour TL_m of 8.2% (Fig. 1). Following three to four hours exposure at two to four % effluent concentrations, the fish experienced a depression of total metabolic rate. At three % effluent concentration, for example, the depression was about 70% of normal rate. An increase in concentration to above four % resulted in a rise in the oxygen consumption rate. An increase to about six % concentration caused an increase in oxygen consumption rate to about 140% normal. Further increases in effluent concentration (above about six %) caused little or no increase in the metabolic rate. It is recognized that these data appear high, although the literature reports little or no metabolic data for subtropical species suitable for comparative purposes (Wohlschlag 1964).

Toxicity can exert more than one type of stress. For example, death can result through different mechanisms depending upon effects caused by different concentrations of the same toxic component (Fromm 1965). Variable response is apparent also from the study by Smith *et al.* (1966) in which routine metabolic rates of test specimens were elevated while active rates were depressed. In our experiment, an inhibiting stress produced by a low concentration (less than 4%) of effluent caused a decrease in metabolic rate as reflected by oxygen consumption. The inhibiting stress probably affected only the degree of spontaneous activity rather than lowering the more stable standard metabolism. Without some record of activity such as swimming speed or observed spontaneous movements, the two cannot be differentiated with certainty (Fry 1957). As the effluent concentration increased to above five %, the fish experienced an increase in oxygen consumption rate. This was due either to an increase in energy necessary to maintain equilibrium or to excitation because of irritation to the fish. In either case, the energy requirement was increased.

Lagodon rhomboides

Results similar to the above were obtained with *Lagodon rhomboides* (Fig. 2) at temperature of 25 C. An increase in effluent concentration to between two and five % caused maximum depression of metabolic rate—down to 82% normal (0.225 mg O_2 hr^{-1} gm^{-1}). Here, also, the lower concentrations appear to exert an inhibitory stress upon the organism. An increase in concentration to that ap-

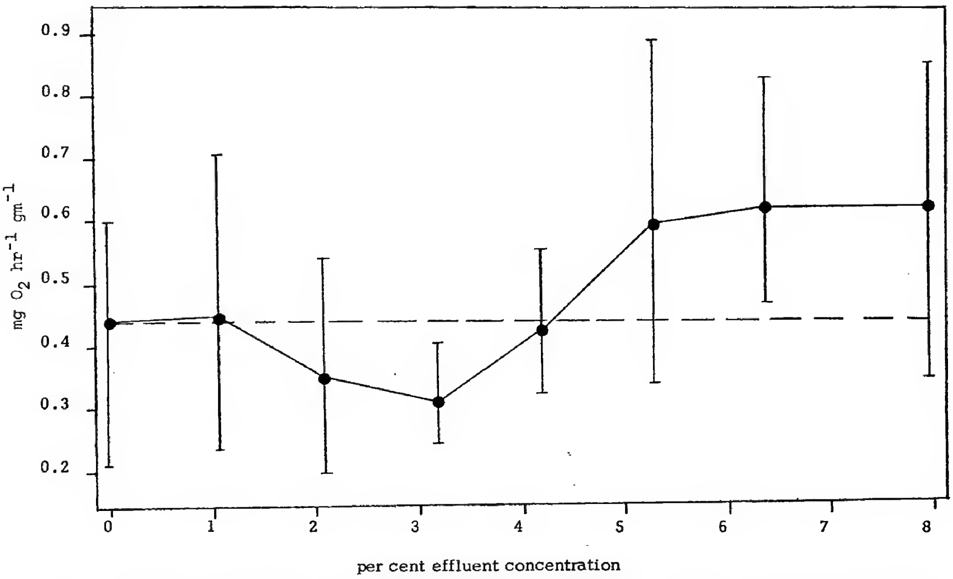

FIG. 1. Oxygen consumption rates of *Cyprinodon variegatus* (mg O_2 hr^{-1} gm^{-1}) in eight concentrations (per cent) of an industrial effluent. Vertical lines give range of measurements and horizontal dashed line is the normal rate (0.44 mg O_2 hr^{-1} gm^{-1}). $TL_m{}^{48} = 8.2\%$.

proaching the TL_m (eight %) brought about a loading stress with the metabolic sponse of increased maintenance costs and oxygen consumption increased back into the normal range. Copeland and Wohlschlag (1967) reported decreased metabolic rates in *L. rhomboides* exposed to water containing unknown pollutants at most temperatures between 10 and 30 C. Results obtained in our experiments are consistent with theirs.

Micropogon undulatus

Micropogon undulatus was found to have a much lower TL_m level than either *Cyprinodon* or *Lagodon*—4.5%. An inhibitory stress from a 1.5 % effluent solution (Fig. 3) brought about a reduction of the oxygen consumption rate to around 71% the normal rate in sea water. Because of the low TL_m, a loading stress was experienced at much lower concentrations than for other species.

Below 48-hour TL_m concentrations, the pattern of response in *Micropogon* corresponded to that in other test species. Oxygen consumption rate measurements conducted in two concentrations (5.0 and 7.5%) above the TL_m value (4.5%) suggest rather erratic responses. However, it should be remembered that even with a single toxicant, responses and cause of death can vary with concentration (Fromm 1965). There is a great variability of response to an effluent containing a mixture of various toxic components because time for biological action of a toxicant is dependent upon:

1. toxicant properties;
2. method of toxicant entry;
3. time for toxicant to reach a site of action from a site of entry;
4. time for action at a site; and
5. time for buildup of an effective internal concentration.

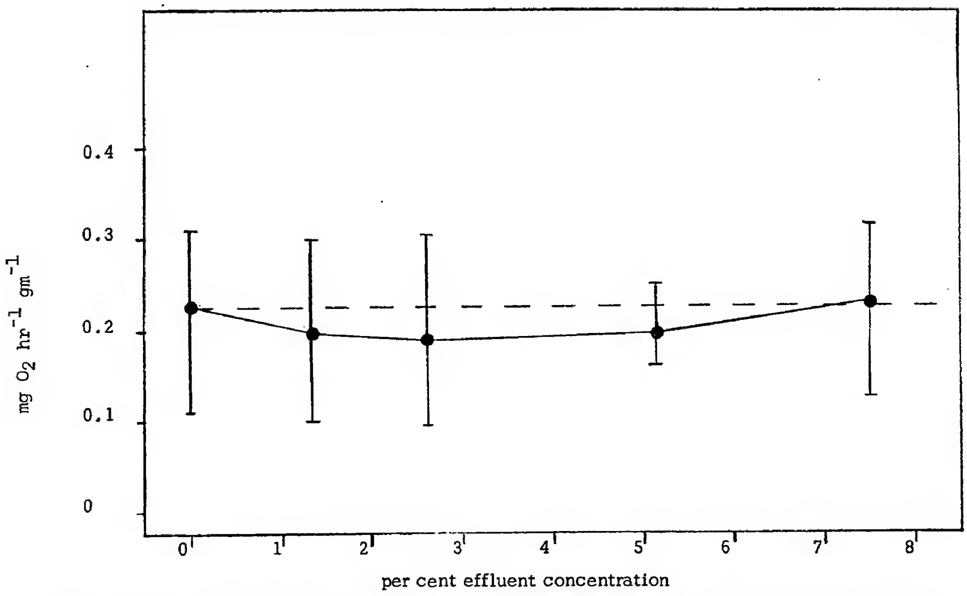

FIG. 2. Oxygen consumption rates of *Lagodon rhomboides* (mg O_2 hr^{-1} gm^{-1}) in five concentrations (per cent) of an industrial effluent. See Figure 1 for explanation. $TL_m{}^{48} = 8.0\%$.

These factors are incorporated into the concept of "biological time" discussed by Wilber (1965). It is apparent that if responses by organisms show variation with different concentrations of an effluent, even greater variation may exist among species.

CHRONIC EFFECTS

Results of the study of chronic effects of a 5% concentration of effluent, having a 48-hour TL_m of 8.2%, upon *C. variegatus* are given in Figure 4. Days holding time is plotted against oxygen consumption rate for control fish in sea water (solid line) and for fish exposed to the effluent (dashed line). These measurements were conducted at 20 C, and the specimens used were collected at 27–29 C. Oxygen consumption rate in the control fish increased for the first four days as acclimation to the lower temperature took place. It is not unusual that acclimation to cold temperatures (5 C) takes up to 20 days in some fish (Fry and Hart 1948). Depression followed and continued at least through the sixth day. Since the fish were not fed during the experiment, this factor possibly imposed a limiting stress which would have caused the decrease in metabolic rate. The organisms exposed to the effluent were inhibited from acclimating to the colder temperature as did those in seawater because of the stress exerted by the effluent. They experienced lower metabolic rates than the control group through the fourth day. After five and six days' exposure, oxygen consumption rates increased respectively to 148 and 210% of control values for the same periods. It appears that chronic

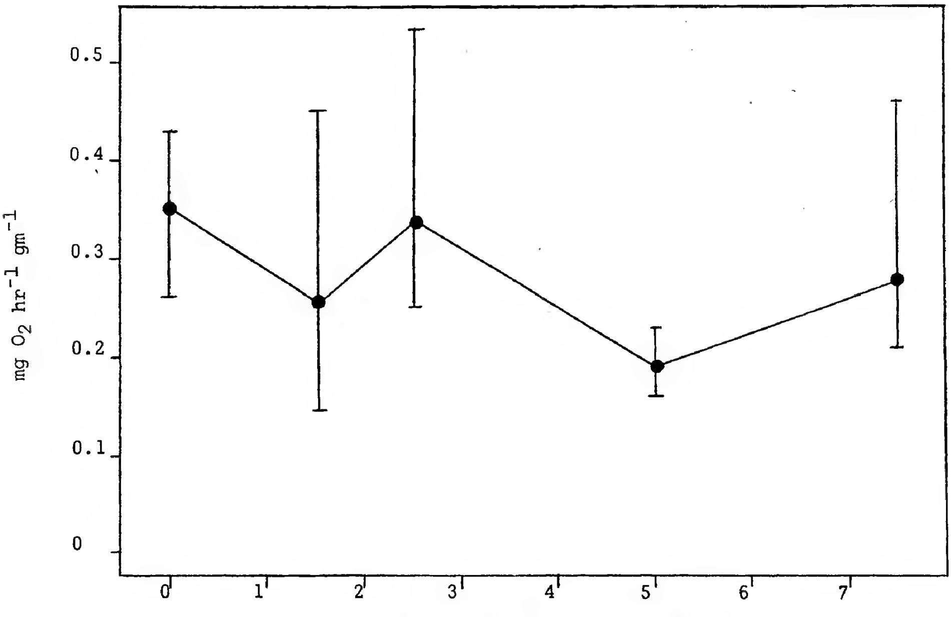

FIG. 3. Oxygen consumption rates of *Micropogon undulatus* (mg O_2 hr^{-1} gm^{-1}) in five concentrations (per cent) of an industrial effluent. See Figure 1 for explanation. $TL_m^{48} = 4.5\%$.

effects of low toxicity levels are similar to effects at higher levels for shorter periods of time.

METABOLIC RESPONSES OF PENAEID SHRIMP

A short experiment was conducted to test for similar metabolic responses to stress conditions among invertebrates. Different responses were observed for the two species of penaeid shrimp used in oxygen consumption experiments (Fig. 5). *P. duorarum* responded to a loading stress from low concentrations, while *P. aztecus* responded to an inhibiting stress. With 1.3 and 2.6% concentrations, *P. duorarum* exhibited increased rates of 169 and 135%, respectively. Under the same concentrations, rates of oxygen consumption in *P. aztecus* were depressed by 55% in both cases. Forty-eight hour TL_m values were also different—3.3% for *P. duorarum* and 4.8% for *P. aztecus*.

Routine metabolic rate of the brown shrimp is higher than that of the pink shrimp in sea water. But the two species are different ecologically, which may account for the physiological differences. While in the bays, the pink shrimp inhabits grass flats and the brown shrimp more commonly inhabits mud-bottomed areas. Organisms living on the grass flats would necessarily have lower metabolic rates or some other mechanism of adaptation to the lower oxygen levels occurring there at night. Near anoxic conditions may be approached during dark hours as a result of high community respiration, especially during highly productive seasons (Odum and Wilson 1962).

It is also possible that the measured control value for *P. duorarum* consisted of

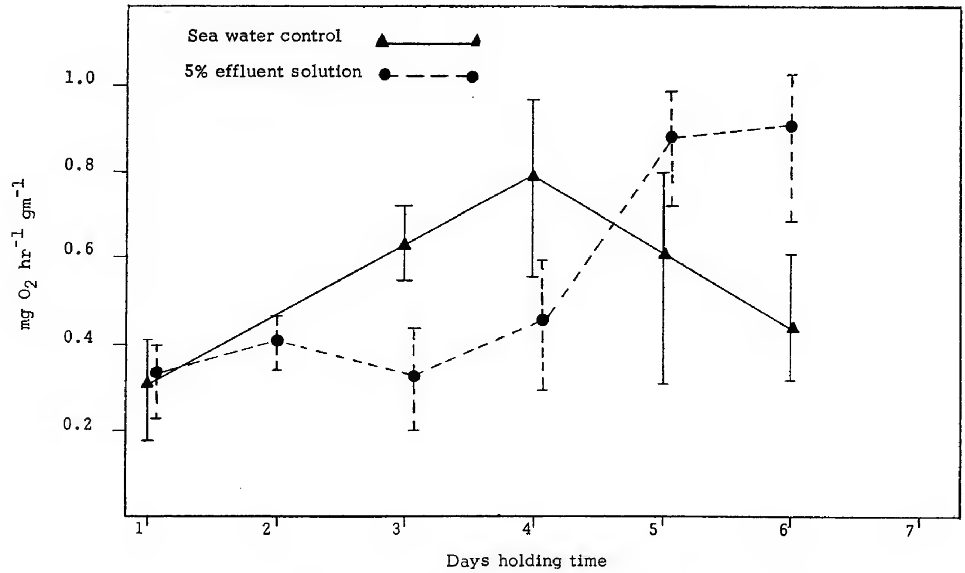

FIG. 4. Oxygen consumption rates (mg O_2 hr^{-1} gm^{-1}) of *C. variegatus* in a five % concentration of an industrial effluent (broken line) and in clean sea water (solid line) for six days holding time. Vertical lines indicate the range of measurements. $TL_m^{48} = 10.3\%$.

a smaller portion of active metabolism than for *P. aztecus*. The effluent, having greater toxicity to *P. duorarum*, resembles a loading stress in much smaller concentrations (less than 1%) than to *P. aztecus*. At the same concentrations it acted as would an inhibitory stress to *P. aztecus*, bringing about a reduction in active metabolism.

ECOLOGICAL IMPLICATIONS

Adverse effects on the estuarine ecosystem from pollution are not always evidenced by large fish kills. Abnormal metabolism due to loading and/or inhibiting stresses from pollution can have far-reaching effects on individuals, populations and the ecosystem. In a system receiving industrial pollution in quantities sufficient to affect organisms, the first response of many species would be failure to frequent such an area or to leave the area in search of a more suitable environment. Among vertebrates, migration of individuals as a response to environmental conditions is common (Slobodkin 1961). This is particularly the case for the Texas coast where a series of interconnecting bays and estuaries differentially receive large volumes of industrial pollution. Commercial harvest of many species has declined to negligible quantities in some bays within the last 10 to 20 years. However, in cases in which escape is not an alternative, other more subtle effects can occur.

The first reactions an organism, especially a fish, would have to a reduced metabolic rate because of an inhibiting or limiting stress would be a reduced capacity to compete with other species, feed adequately, or protect itself from predators. A limiting stress would also reduce its capacity to cope with other ex-

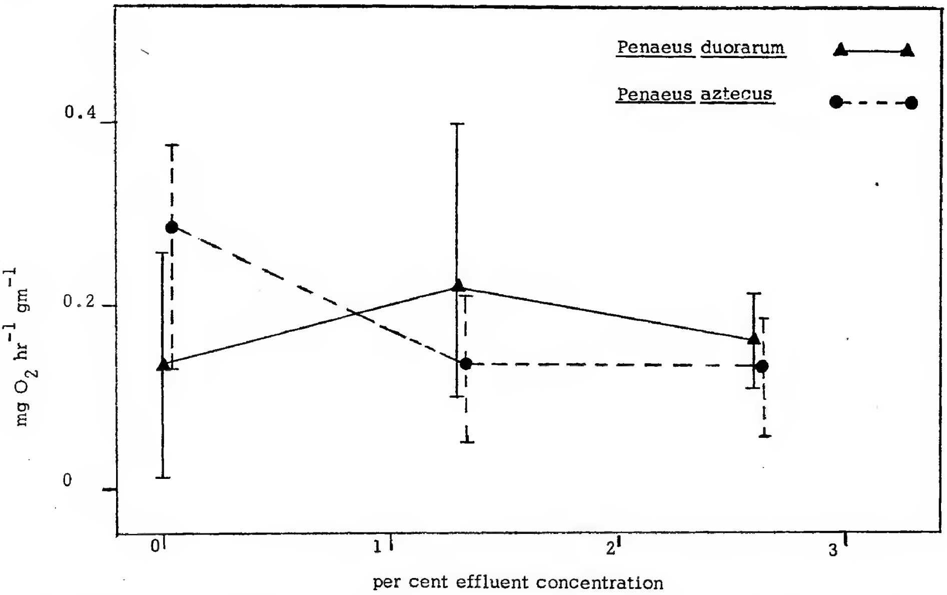

FIG. 5. Oxygen consumption rates for *Penaeus duorarum* (solid line) and *P. aztecus* (broken line) in different concentrations of an industrial effluent. See Figure 1 for explanation. $TL_m{}^{48} =$ 3.3% for *P. duorarum* and 4.8% for *P. aztecus*.

treme environmental changes otherwise within its range of adaptation. Sheppe and Adams (1957) reported a decrease in capacities for survival under low temperature and starvation in animals with an additional stress from parasitism. A metabolic rate reduced to below standard rate would bring about a rapid decline in general condition of the organism making it more susceptible to diseases. Death could ensue within a short time.

A slower growth rate would result from a slightly decreased metabolic rate or from reduced activity levels (reduced feeding rates). Smith *et al.* (1966) reported evidence for decreased growth rates in walleyes under long-term exposure to suspended conifer-groundwood fiber. The population begins to be adversely affected, even though there may be no deaths of organisms as a direct result of stress conditions. Organisms with slower growth rates may not reach sexual maturity within the time necessary to maintain normal population density and age-class composition. The fundamental dependence of normal population mortality and natality upon age distribution are discussed by Allee *et al.* (1949) and Lotka (1925). Thus, when a change in population structure occurs, some populations are able to maintain themselves under the new conditions and others cannot.

It is conceivable that life-span for fishes under stress conditions would be significantly reduced. Ursin (1965) discussed cyclic weight variation in old fish as pertaining to a possible cause of mortality. It seems that once past a peak, the condition of a fish decreases with age because the theoretical maximum weight is reached prior to attainment of the theoretical maximum length. Thus, an old fish may continue to increase his length without increasing his weight, with his overall condition being reduced. Improvement is made each year during the growth season, but the total annual loss becomes greater with age. The implications are that the process proceeds more rapidly when a stress is exerted upon the fish. When the metabolic rate is altered, annual improvement is not made or the cyclic variation is made greater. In either case, death ensues at an age earlier than normal, and the population composition will be altered.

In a population of fish exposed to a loading stress (*i.e.*, from pollution), maintenance costs of individuals are increased and death may result from an inability to satisfy higher energy requirements. Since many species of fish feed at a maximum rate allowed by food density and behavioral patterns under normal conditions, condition of the fish suffers when maintenance costs are increased without allowance for a more efficient or an increased feeding rate. Thus, the emaciation of fish in long-term exposure to sublethal concentrations of toxicants (Graham 1963), even when feeding and behavior appeared normal, is explained.

An abnormally high metabolic rate from a loading stress may be a lethal factor, *per se*, even before toxicity proves fatal. Black (1958) reported that death may occur in fish immediately following severe activity. The oxygen and carbon dioxide carrying capacity of fish blood is altered by the accumulation of products of fatigue (Black 1957a; 1957b; 1957c). It is plausible that any factor necessitating the rapid release of muscular energy might have the same effect. A fish with abnormally high metabolism, due to irritation or excitation from some toxic compound, may soon die from the lethal action induced by fatigue even though physiological equilibrium may have otherwise been maintained against the toxicant.

Cost to the ecosystem by stresses upon populations of aquatic organisms may be even more subtle than the mechanisms discussed above. Metabolic imbalances may be severe enough only to limit reproductive energies while individuals are otherwise little affected. Toxicity may be confined to a particular embryonic or juvenile stage. Perhaps no toxic effects are evidenced through direct reactions by populations of fishes or invertebrates. However, in a balanced ecosystem, to disturb a population of organisms at any trophic level is to upset the normal flow of energy through the food chain. There is evidence that energy flow pathways are altered or replaced in ecosystems subjected to environmental stress (Copeland 1965). Such changes brought about in a polluted marine or estuarine system can be recognized by monitoring abnormal community metabolism (Odum *et al.* 1963; Copeland 1966).

When a natural system is disturbed, the communities replacing those being removed are not always as productive in terms of organic matter harvestable by man. Biological changes in polluted aquatic systems are discussed by Hynes (1960). Reduced species diversity in polluted marine systems have also been noted (Copeland 1966; Odum *et al.* 1963). It is of interest to note that in experiments conducted here, the species of greatest value to man—the Atlantic croaker and penaeid shrimp—had lower tolerance for the industrial effluent used than did those species of much less value—*Cyprinodon* and the pinfish. It is probable that should the shrimp industry of the Texas coast to be destroyed through man's negligence, the shrimp populations would be replaced with populations of organisms even less useful to man than *Cyprinodon* or the pinfish.

Millions of gallons of waste similar to that used here are being discharged daily into estuaries and bays along the Texas coast. Obviously these pollutants have a severe impact upon organisms dwelling in those areas. In light of data and discussion presented it is clear that one does not need to have gross apparent effects, such as obvious fish mortality, to show that drastic changes are being made in these natural systems. Present pollution control measures are inadequate to cope with the more subtle but still severe effects that pollution is having upon areas of large economic value to man.

SUMMARY

1. Measurement of oxygen consumption rates is a suitable method for detecting metabolic responses in aquatic organisms to sub-lethal and/or chronic toxicity from industrial pollution.

2. Three species of fishes from Texas estuarine waters, *Cyprinodon variegatus*, *Lagodon rhomboides*, and *Micropogon undulatus*, were found to have similar metabolic responses to a petrochemical waste. The response is to an inhibitory stress in low concentrations, and a loading stress in concentrations approaching the 48–hour TL_m value.

3. Two species of penaeid shrimp responded differently to exposure to a petrochemical waste. *P. aztecus* experienced a decrease in routine metabolic rate, while *P. duorarum* had an increased rate. The responses were attributed to differences in physiological requirements which accounted for ecological differences between the species.

4. The response by *C. variegatus* to chronic exposure to the petrochemical waste resembled a response to short-term exposure to more toxic concentrations. An inhibiting stress in early stages of exposure gave way to a loading stress in the later stages of response.

5. The possible long-term effects of environmental stresses (pollution) upon a population are discussed. There may be a reduced growth rate, life-span, and/or reproductive capacity; population age and sex distributions may be disturbed. All these factors lead to failure for successful exploitation of the former niche.

ACKNOWLEDGMENTS

The senior author was supported by the U.S. Public Health Service Environmental Sciences Interdisciplinary Training Grant No. 5T1-ES-65-02 to The University of Texas, Department of Civil Engineering. Appreciation is extended to Mr. Roy W. Spears, Texas Parks and Wildlife Department Marine Laboratory, Rockport, Texas, who provided data on chemical components of the petrochemical waste; Drs. Bassett Maguire and Earnest F. Gloyna who criticized the manuscript and provided many helpful hints; Mr. William H. Gillespie, who prepared the drawings; Mr. Herman Moore and Mr. Elgie Wingfield, who piloted the *M/V* Lorene; and Mr. Frank Moseley, Dr. Dave Lane, Mr. Woody Elston, and Miss Patti Moehlman, who provided numerous suggestions and helped with collecting. We appreciate the advice and criticisms of Dr. D. E. Wohlschlag and Mr. J. N. Cameron throughout the study and construction of the manuscript.

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ASSESSMENT OF A LOW LEVEL STRESS ON THE RESPIRATORY METABOLISM OF THE PINFISH *(LAGODON RHOMBOIDES)*[1]

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ABSTRACT

Respiratory metabolism of the pinfish was determined by measuring oxygen consumption in an enclosed plastic chamber that could be rotated. By means of multiple regression equations the observed oxygen consumption rates were related to body weights, temperatures, and swimming velocities for fish acclimated in both unpolluted and slightly polluted waters over the temperature ranges of 10–20 C and 20–30 C and at the single temperature of 20 C. Waters were kept at relatively high levels of dissolved oxygen. Under normal conditions the pinfish seems to have quite high rates of oxygen consumption compared to temperate species.

In all cases the slightly polluted water caused a depression of respiratory levels. There was the lowest degree of depression at or near 20 C. For small fish the response to temperature was nearly linear from 10–30 C; depression of respiration was relatively uniform for both resting fish and fish swimming at 10 m/min. For larger fish at 10 C, there was a pronounced depression of respiration in polluted waters, especially for swimming fishes; at 30 C the depression was marked for both swimming and resting fish. For larger swimming fish, the possibility of metabolic loading is discussed at both the 10 C and 30 C levels. Statistically, the greatest variability in oxygen consumption rates occurs at the lowest temperatures, and least at 20 C. The swimming-oxygen consumption rate coefficients are highest for the lower temperature ranges.

The great metabolic depression due to stress on larger fishes at low temperatures is discussed in terms of winter kills that tend to affect larger fish most severely. The possible effects of thermal

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[1] This study was supported in part by National Science Foundation institutional grants NSF-GU1498 and NSF-GU1963.

[2] University of Texas, Graduate Fellow.

"pollution," whereby the winter temperatures could be raised artificially, is appraised in terms of relatively high maintenance requirements under stresses of high and low temperatures in the presence of stresses like those induced by pollution. The results of slow growth rates on population dynamics are also evaluated in terms of adverse survival rates.

INTRODUCTION

The purpose of this study is to gain some knowledge of the normal respiratory metabolism levels compared to the subtle influence of these levels by a very low level of pollution for the common temperate to subtropical estuarine fish, *Lagodon rhomboides*.

Although the coastal lagoon-estuarine systems are very highly productive in a biological sense, these waters along the coast of Texas support relatively few species (Hoese 1958; Gunter 1945). The seasonal changes in salinity and temperature are great and the diurnal changes in dissolved oxygen during the summer are pronounced in the shallower waters. The reasons for the paucity of species that accompany the high biological production rates have been discussed in terms of these environmental changes by Hedgpeth (1966). The metabolic effects caused by slight levels of pollution have been but little investigated for subtropical fishes, although there is a large literature on the effects of pollution on fishes of more temperate waters. There is relatively little known about the metabolic levels of fishes in unpolluted subtropical waters. However, there is a large literature on respiratory metabolism of other fishes as indicated in the reviews by Fry (1957; 1964) and Winberg (1956) and by many other individual investigators more recently.

In order to avoid confounding the effects of pollutants with the secondary effects of low dissolved oxygen levels produced by the biological or chemical oxygen demands of the pollutants, this study has proceeded under the rationale that respiratory measurements made under conditions of high dissolved oxygen levels would yield an evaluation of the effects of the pollutants alone. The choice of the pinfish for study is based on its easy availability and widespread occurrence along the U. S. middle Atlantic coasts continuously along the coastal waters to Yucatan in a wide variety of habitats; general biological and ecological information on the species is abundant (Caldwell 1957).

It is a particular objective of this study to assess the stress effects of pollution at levels far below ordinary toxic levels over a range of sizes, temperature levels and swimming velocities. The implications and assessments of environmental stresses have been reviewed by Brett (1958), Kinne (1963; 1964), Doudoroff and Warren (1965), among others. At low levels, the definitive effects of long term pollution are not well understood, although the general effects are known to involve population reduction or elimination as well as impairment of growth rates.

PROCEDURE

The pinfish utilized were captured in the Aransas Pass area near the Gulf of Mexico (Lat. 27° 50′ N., Long. 97° 03′ W.) during the colder February-March period of the year at temperatures of 13–16 C and salinities around 32–35 ppt. The fish ranged in standard length from 87 to 203 mm and in weight from 16 to

221 g. Unpolluted waters were obtained from the Aransas Pass during incoming tidal flows; polluted waters of similar salinities and temperatures were obtained from the Corpus Christi turning basin and diluted one-half with unpolluted water. The fish were placed in a 463–liter insulated aquarium under controlled temperature conditions and under conditions of aeration at near-saturation levels.

Fish were held under fasting conditions in the aquarium at temperatures near 10, 15, 20, 25 and 30 C for 3-day periods at ±0.05 C before each of the oxygen consumption measurements, as described by Copeland and Wohlschlag (1967). In all cases, fish were held for three days at any one of the 5 C intervals before a 1-day change to the next 5 C level. Six oxygen consumption determinations (5 in one case) were made at each temperature level for each of the polluted and unpolluted series.

Because the metabolic rates in polluted or unpolluted waters appeared to be about the same at the 20 C level, an additional 10 fish in polluted, and 9 fish in unpolluted, water were utilized for a supplementary experiment in early April when seawater temperatures had warmed to 18–20 C.

Metabolism was measured in terms of oxygen consumption by the individual fish in an enclosed circular transparent 10.3-liter plastic chamber. Oxygen levels of water in the chamber were determined initially and at three successive 15-minute intervals. A simple linear regression through the 4 points was calculated to determine the rate of oxygen consumption in mg 0_2 consumed per hour. During the 45-min period of a run, the swimming velocity of the fish was measured in m/min at whatever velocity the fish would swim consistently. Velocities were recorded from zero to 16 m/min. Temperatures were recorded to ±0.01 C. The weight of each fish was recorded to ±0.1 g after each run. The general procedure has been described in detail by Wohlschlag (1962a).

To analyze the data by the multiple regression method, the standard computational techniques were used (*e.g.*, Goulden 1952) by relating the log oxygen consumption rate to log body weight, temperature and swimming velocity. For the two small groups of data at 20 C the temperature variable was eliminated. Because the temperature response was not linear over the entire 10–30 C range, the other regressions were limited arbitrarily to 10–20 C and 20–30 C.

RESULTS

Regression of the oxygen consumption measurements in relation to the independent variables is useful in the form:

$$\hat{Y} = a + b_w X_w + b_t X_t + b_s X_s;$$

where

$\hat{Y}$ is the expected log mg 0_2 consumed per hour,

a is a constant,

b_w is a partial regression coefficient, the increase in $\hat{Y}$ per unit log weight increase at constant temperature and swimming velocity,

X_w is the log weight in grams,

b_t is a partial regression coefficient, the increase in $\hat{Y}$ per C increase in temperature at a constant weight and swimming velocity,

X_t is the temperature in C,

b_s is a partial regression coefficient, the increase in $\hat{Y}$ per each m/min increase in swimming velocity, and

X_s is the swimming velocity in m/min.

For the equation pertinent to the series at 20 C the form is:

$$Y = a + b_w X_w + b_s X_s.$$

The equations, with calculated figures to four places are in the schedule below:

Polluted waters:

(1) 10–20 C range, $\hat{Y} = -1.4060 + 0.7511 X_w + 0.0746 X_t + 0.0182 X_s$;
(2) 20–30 C range, $\hat{Y} = -0.0094 + 0.7639 X_w + 0.0043 X_t + 0.0123 X_s$; and
(3) 20 C re-run, $\hat{Y} = -0.1370 + 0.8636 X_w + \ldots \qquad + 0.0124 X_s$.

The equations, with calculated figures to four places are in the schedule below:

Unpolluted waters:

(4) 10–20 C range, $\hat{Y} = -0.8908 + 0.9389 X_w + 0.0287 X_t + 0.0302 X_s$;
(5) 20–30 C range, $\hat{Y} = -0.3397 + 0.7855 X_w + 0.0178 X_t + 0.0108 X_s$; and
(6) 20 C re-run, $\hat{Y} = -0.1208 + 0.8828 X_w + \ldots \qquad + 0.0126 X_s$.

Also available from the calculations of equations (1), (2), (4) and (5) are appropriate statistics to measure the over-all correlation, $R_{Y.wts}$, among the variables, the standard error for the over-all regression, $s_{Y.wts}$ and the standard errors of the individual partial regression coefficients s_{bw}/s_{bt} and s_{bs}. These statistics and probabilities are in Table 1. Too few degrees of freedom exist for adequate analysis of equations (3) and (6).

From the above equations, Figure 1 gives an instructive comparison of oxygen consumption rates for 10- and 100-gram fish, for fish in polluted and in unpolluted water and for resting fish or those swimming at 10 m/min. In Figure 1 the values at 10 C and 30 C are from equations (1), (2), (4) and (5); the values at 20 C are from (3) and (6). Ten- and 100-gram values are arbitrary and illustrate a difference of an order of magnitude. At 10 grams, the extrapolation below the sizes of the smallest fishes is slight and not unreasonable; at 100 grams, the size is of minimal commercial or recreational interest. The zero swimming velocity represents a minimal ecological requirement; the 10 m/min value is a reasonable upper level that can be maintained for at least 45 minutes without stimulation under the most adverse conditions studied.

DISCUSSION

In the crude presentation of some of these data (Copeland and Wohlschlag 1967) on the basis of oxygen consumption rate per kilogram without consideration of body size or swimming rates, it is clear that there is a pronounced metabolic depression due to pollution at the lowest temperatures. To be emphasized are the facts that these data are for waters near oxygen saturation levels and near normal levels of salinity and that altered oxygen consumption levels are primarily under the influence of pollution and not salinity or oxygen levels.

From the equations above and Figure 1, it is apparent that pronounced me-

TABLE 1

Regression statistics of *Lagodon rhomboides*. Multiple regression equations with probability (P) values.

Equation	Degrees of freedom	Standard error of estimate	Multiple correlation		Standard errors of estimate for partial regression coefficients					
			R	P	Weight, b_w	P	Temperature, b_t	P	Velocity, b_s	P
(1)	13	0.0738	0.97	<0.001	0.1378	<0.001	0.0054	<0.001	0.0128	>0.10
(2)	14	0.0710	0.94	<0.001	0.0749	<0.001	0.0048	>0.10	0.0154	>0.10
(4)	14	0.1091	0.91	<0.001	0.1529	<0.001	0.0063	<0.001	0.0152	>0.05
(5)	14	0.0514	0.97	<0.001	0.0611	<0.001	0.0033	<0.001	0.0067	>0.10

tabolic differences exist between fish in slightly polluted and unpolluted waters.

Steed and Copeland (1967) describe the nature of the petrochemical waste water near the source of the effluent in the Corpus Christi turning basin and note its level of toxicity. Because the waters in this study were from the more open portions of the basin, the salinities were near normal, and the influence both of other domestic sewage and of effluents from nearby agricultural products processing activities was also present. The undiluted waters would kill about 5–10% of pinfishes at the ambient temperatures of 13–15 C in the five-day period. When

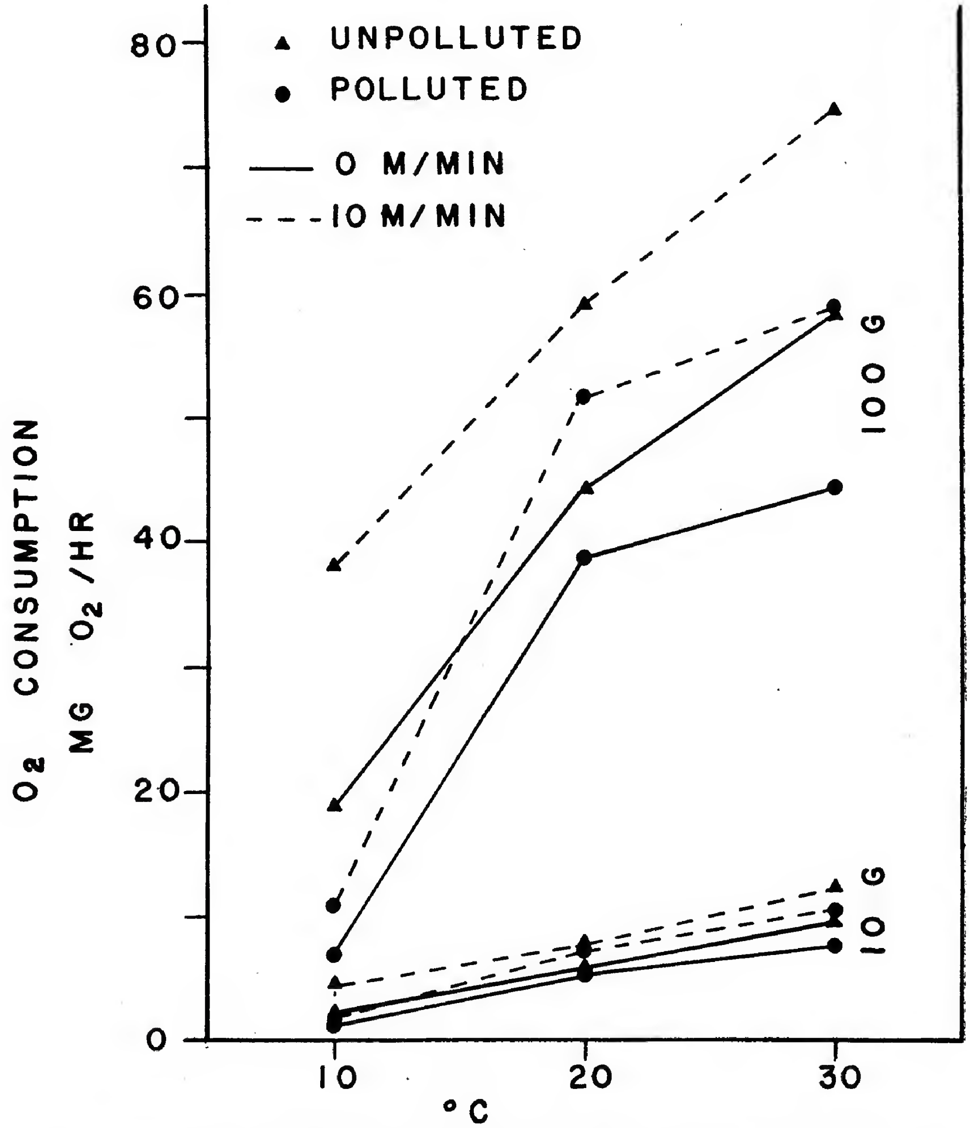

FIG. 1. Oxygen consumption levels of pinfish calculated for 10- and 100-gram fish, for polluted and unpolluted water and for zero and 10 m/min swimming velocities. (See text for equations.)

diluted 50% with unpolluted sea water, however, the pinfish would survive for periods of at least two weeks without mortality. Under these circumstances of sublethal levels of pollution from mixed sources, normal salinities and relatively high levels of dissolved oxygen, the study of tolerance limits would not be particularly pertinent unless the fish could have been studied over considerable periods of time with an appropriate feeding regime.

Although there appears to be a lack in the literature of respiratory metabolism data for subtropical fishes, the levels of metabolism in this study agree with those fishes in general (Wohlschlag 1964) and with the levels recently determined by Steed and Copeland (1967) for the pinfish and two other fishes from the Texas coast. However, the data from Steed and Copeland (1967) are based on shorter times of temperature acclimation and relatively acute three- to four-hour acclimation periods to the polluted waters. At zero velocities, the level of oxygen consumption would appear to be higher ("routine") than the lowest possible resting ("standard") levels according to experiments in progress. Fry (1957) notes that the differences between these two levels can be due to "spontaneous" activity in which a large amount of non-locomotor energy can be expended. Smit (1965) also notes that considerable differences exist between energy requirements for spontaneous, non-oriented swimming and forced swimming. Brett and Sutherland (1965) discuss the upward displacement of oxygen consumption rates by the pumpkinseed (*Lepomis gibbosus*) at zero or very low velocities in terms of "aggressive behavior" which can increase oxygen consumption about two or three times the standard rate. There is substantial literature that indicates respiratory metabolism levels are more often routine than standard. Furthermore, compilations of respiratory metabolism levels for many species of fishes indicate quite clearly that both the nature of the species and their ecological characteristics must be considered (Fry 1957; Winberg 1956; Wohlschlag 1964) in order to explain differences among the levels.

Despite all precautions, pinfish tend to remain under laboratory conditions in a fairly irritable state. They do not become placid as do many species, even under darkened, undisturbed conditions. The species is quite aggressive in the normal environment. It tends to feed more or less continually during daylight hours (and possibly at nighttime); it seems to exhibit rather more excitability and non-locomotor activity than many of the species commonly utilized for laboratory studies. Accordingly, the pinfish would normally be expected to have a relatively high respiratory metabolism under standard conditions. Because of the different nature of fishes under different environmental situations the useful physiological concept of a standard rate for each set of controlled physical conditions is open to question in ecological terms. For ecological and behavioral reasons the rates determined in this study at zero velocities are somewhat higher than expected standard rates, but these higher rates are tentatively considered to be the more realistic in terms of what the fish is and does in the natural environment. Whether or not there can be a sensible "ecological standard" rate equivalent to a "physiological standard" rate will depend upon the outcome of much additional research.

It is generally recognized that low dissolved oxygen levels associated with pollution depress locomotor activity and growth, with the relatively greater depression at the higher temperatures (Doudoroff and Warren 1965), but in this study (except for evidence of growth depression) the results are the same even at relatively high dissolved oxygen levels. Under saturation conditions, walleye growth depression is evident under stress caused by wood-fiber wastes, according to data from Smith, Kramer and Oseid (1966). From data of MacLeod and Smith (1966) on the fathead minnow with the same pollutant, there is a similar stress that causes impaired oxygen uptake and swimming performance.

Brett (1958) lists several kinds of stress effects: lethal, limiting, inhibiting and loading. The more subtle inhibiting, or depressing, effects on metabolism and growth of subtropical species like the pinfish are at present little understood over the wide range of temperature and other environmental variables. Likewise, there is little ecologically pertinent data available to evaluate the long term effects of loading, although it might be assumed that the eventual effect may be the same sort of depression of metabolism and growth as in the case of inhibition. Prolonged loading would logically cause death.

From the separate equations and the Figure 1 plots, the effects of temperature on oxygen consumption are especially pronounced at both the lower and upper levels. Slightly below 10 C and above 30 C there appears to be the beginning of lethal levels, and the depression of oxygen consumption rates becomes *unmeasurable* because a high degree of morbidity among the fish occurs. It is especially important to note that for the smaller fish (*e.g.*, at 10 g) there is a rather uniform and linear rise in oxygen consumption with temperature for active and inactive levels and for polluted and unpolluted waters; while for the larger fish (*e.g.*, at 100 g), there is a much more obvious depression in the polluted waters at both the 30 C and the 10 C extremes. As represented by the points at 10 C, 20 C and 30 C, the line for the 100-g fish in unpolluted waters is in reasonable agreement with the form of metabolism-temperature relationships for fishes in general. The weight coefficients are within the usual ranges and there is no apparent reason for the differences in the various equations. Over the range of fish sizes and activities, there appears to be a very slight statistical variation among the oxygen consumption measurements at 20 C, while there is much greater variation at other temperatures, especially at 10 C. The phenomenon of greater metabolic variability at lower temperatures is apparently real, as noted by Wohlschlag and Juliano (1959) and Wohlschlag (1963; 1964). From Table 1, there is evidence that the standard errors are greater in the 10–20 C range for the overall regressions and for the partial regression coefficients of weight and temperature.

The ecological and zoogeographical significance of the metabolic depression at the temperature extremes, while not fully understood, has some important implications regarding survival of populations in terms of both laboratory and field observations. At the lowest temperatures, there is a very pronounced depression of oxygen consumption rates for the larger fish in polluted waters to the extent that swimming fish have lower rates than non-swimming fish in unpolluted waters. According to Brett (1958), loading reduces the temperature range over which maximal activity is possible, which, in effect, reduces the "scope for activity." Observations in the laboratory indicate that the larger fish suffer severe stress after swimming for the 45-minute period when they tend to become irritable or even slightly moribund. Inasmuch as the same explanation could not be made for the smaller specimens at 10 C, the depression of the line in Figure 1 for 10-g fish swimming at 10 m/min in polluted waters below the corresponding line for non-swimming fish in unpolluted waters is quite likely the result of a vagary in statistical variation. However the depression for the larger fish swimming in polluted water below the level for non-swimming fish in normal water is a rather clear indication of "loading" which could not persist indefinitely. This kind of metabolic loading is quite possibly involved in the explanation of winter kills that occasionally occur along the Gulf and southern Atlantic coasts of the U.S.

Storey and Gudger (1936), Storey (1937), Gunter (1941), Gunter and Hildebrand (1951), Simmons (1962), among others, have reported the effects of sudden temperature depressions to less than about 8 C in the shallow waters. The usual observations are that larger numbers of larger fish are stunned or killed and that there is a tendency for the larger fish to move to deeper, and presumably warmer, waters if the temperature drop is not too sudden. If the data on metabolic depression for larger fish in Figure 1 are realistic it is likely that very little energy would be available for larger fish to cope with such stresses. With further stress and possibly loading in slightly polluted waters, the explanation of why slightly polluted waters at even moderately low temperatures become restrictive to the distribution of some species is tenable, even though the species could normally tolerate much lower temperatures for short periods of time. Movement also requires more energy at lower temperatures as indicated by the oxygen consumption-swimming velocity coefficients, which are considerably higher at lower temperatures. Although these swimming coefficients are not statistically significant for the pinfish experiments they are realistic and of the same order as those summarized for a variety of fishes at temperate to polar temperatures by Wohlschlag (1962b).

At the 30 C level for the larger fish there is a relatively small drop in the rate of increase of oxygen consumption with temperature in unpolluted water, but the degree of depression is considerable in the polluted water. At 10 m/min swimming velocity in polluted water there is about the same level of oxygen consumption as there is for resting fish in unpolluted water, which is evidence for loading among active fish under stress conditions. At 30 C the larger and more active fish were visibly in rather poor condition after a 45-minute run in the polluted water. While the fish could be raised temporarily to temperatures above 30 C, if handled with great care, the fish in the polluted waters above 30 C tended to become moribund and die during the usual acclimation period. It is believed that the pinfish in nature cannot regularly tolerate temperatures much above 30 C for many days.

In the normal course of annual temperature variations, the shallow Gulf coastal waters have extremes to about 8 C and somewhat above 35 C at least for short periods of several days, while in the nearby deeper waters the extremes would be somewhat less pronounced. Caldwell's (1957) studies on the pinfish indicate that the species has a very slow growth rate under the normal environmental circumstances, with growth to about 80 mm the first year and with growth to largest sizes near 300 m in about 7 years. Preliminary studies indicate that growth may be even slower in the Port Aransas area. Gunter's (1950) information indicates that species having distributions from the Atlantic around to the warmer Gulf coasts quite commonly have slower growing members and smaller individuals along the Gulf coast. If the inhibiting stress and loading depression effects on oxygen consumption rates were greater for the larger fishes in the warmer waters, it is quite apparent that the total energy required for maintenance alone could be so great that less energy would be available for growth; especially since metabolic requirements for growth are counterbalanced by the requirements for maintenance at maximum size. The greater the stresses and the greater the maintenance costs are, the less energy is available for continued growth, and the less would be the maximum size. On the basis of the degree of metabolic depression, the data would indicate that depression due to pollution would be minimal around 20 C, a temperature that occurs only briefly in the autumn and spring. Other stresses like those imposed by swimming requirements also have more pronounced depressing effects for larger fishes at other than optimal temperatures (Brett *et al.* 1958; Brett 1964). Optimal salinities, not considered in this study, can be of great importance in light of recent studies by Kinne (1963; 1964) who notes that maximum growth, food intake and energy conversion can occur under conditions of maximum salinity acclimation which is possible at near-optimum temperatures. Although not pertinent to this study, it is essential to point out that an even more important stress effect on fish growth and maintenance would be the result of low dissolved oxygen levels that occur naturally or in conjunction with pollution.

The effects of stresses in depressing metabolism are also important in considering the dynamics of populations whose growth rates are correspondingly lowered. As pointed out by Copeland and Wohlschlag (1967) and earlier by Ricker (1963), there can be a long term effect of slightly increased mortalities that result in a reduced chance for survival to the larger size ranges and in reduced populations generally; without increasing mortality rates, but by decreasing growth rates, individuals within a population would be subjected to the natural mortality rates for a longer period with the same effects. In a recent essay, Hedgpeth (1966) discusses related reasons why the estuarine ecosystem tends to have a less varied biota than the sea on grounds of relatively rapid short term fluctuations of environmental variables in estuaries that do not permit the existence of continuous, sufficiently optimal conditions for the maintenance of a species.

The results of this study, based on fishes taken during the coldest part of the year, can also have some practical application in cases where environmental alteration would involve warming of waters to more optimal temperatures. In the winter, at least, warming of natural waters by discharges from industrial heat exchange systems could be beneficial to populations like pinfish, assuming that the entire ecosystem would be similarly benefited by more optimal temperatures. On the other hand, excessive heating of waters by thermal "pollution" during other seasons would tend to be deleterious.

ACKNOWLEDGMENTS

Facilities at the Marine Science Institute were utilized and many of the staff contributed their time and ideas. We are grateful for the technical work of Mr.

Carl Meadows, who helped with the construction of much of the equipment. The ideas and constructive criticism of Dr. B. J. Copeland and Mr. D. L. Steed have been especially helpful.

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REFLECTING CELLS OF THE ELASMOBRANCH TAPETUM LUCIDUM

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ABSTRACT

The reflecting cells of the tapetum lucidum of selachians are described, their shapes, disposition and contents. They are ellipsoidal discs which, except in the middle of the fundus, are arranged regularly and concentrically about the center of the eye. The cells contain reflecting crystals; these are elongate flat hexagons whose long axes lie parallel to the inner surface of the eye. Reflecting cells and crystals of *Squalus acanthias* and *Scyliorhinus canicula* have been examined by electron microscopy. The reflecting cells are shown to contain regularly arranged stacks of reflecting platelets (crystals), each being enclosed in a membrane-lined sac. Parameters have been determined for the number of superposed crystals in a cell (12–13), spacing (generally 300–400 nm) and size of crystals including thickness (between 30–70 nm). The high reflectivity of the tissue is produced by the summed reflections at the surfaces of many superposed crystals, and the factors that give rise to white luster and highly unsaturated spectrum reflectance are discussed.

INTRODUCTION

A tapetum lucidum chorioideale occurs in the eyes of most chondrichthian fishes which have been examined, including both selachians and chimaerids (Nicol 1964). This tapetum lucidum, of signal appearance, lies in the inner region of the chorioid layer, immediately external to the choriocapillaris. It is made up of thin flat cells containing reflecting crystals; there is much evidence that the crystals are composed of guanine (Pirie and Simpson 1946; Nicol 1963).

The organization and function of the tapetum lucidum of chondrichthians have been described previously in several papers (Denton and Nicol 1964; Nicol 1964), which also review earlier literature. The present paper is a continuation study and describes the arrangement of the reflecting lamellae or crystals in the reflecting cells, as revealed by light microscopy and by electron microscopy. In the opening sections a summary of the cyto-architecture of the tissue is presented for the convenience of the reader.

ORGANIZATION OF THE TAPETUM LUCIDUM

The tapetum lucidum consists of a layer of thin flat reflecting cells, plate-like in form, whose inner flat surfaces (or planes) face the retina. The reflecting cells are differently oriented in various parts of the eye. In the central fundus the plane surfaces of the cells lie parallel to the retinal surface; the slope increases and becomes maximal near the periphery of the retina.

The chorioid layer external to the reflecting layer contains black pigment cells, and there is a layer of melanophores at the base of the reflecting cells. The pigment is probably melanin (Fox and Kuchnow 1965).

A highly diagrammatic representation of the organization of an occlusible tapetum lucidum, seen in section, is presented to show the orientation and relationships of the several constituent cells and tissues (Fig. 1).

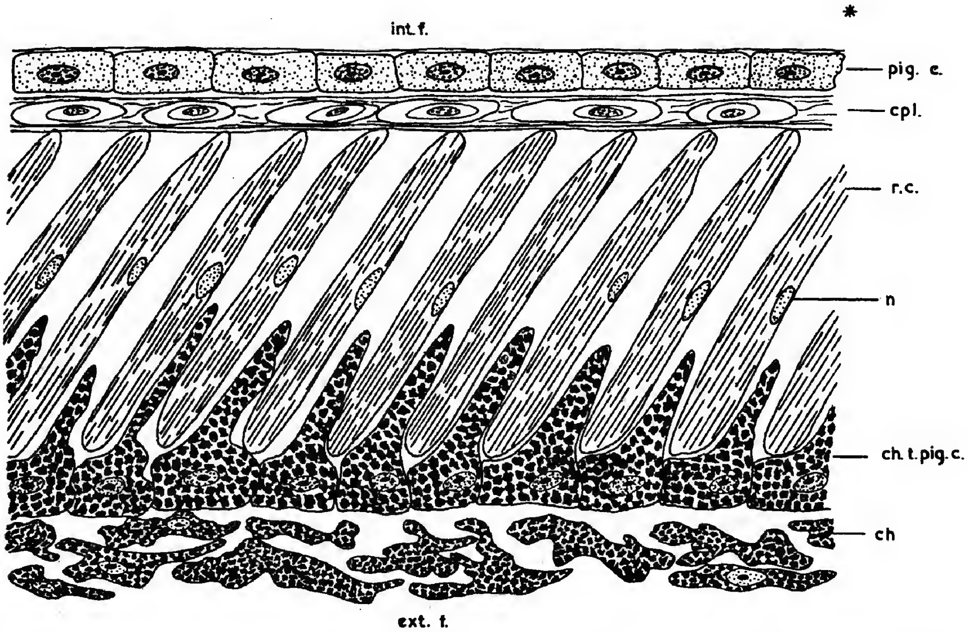


FIG. 1. Diagrammatic representation of the tapetum lucidum of a spur dog, as seen in transverse section. ×1,000. ch, pigment of the chorioid layer; ch. t. pig. c., chorioid tapetal pigment cell; cpl., choriocapillaris; ext. f., external face of tissue; int. f., internal face of tissue; n, nucleus; pig. e., pigment epithelium (not pigmented); r. c., reflecting cell of the tapetum lucidum. The center of the eye is to the right (direction of central fundus indicated by asterisk); the periphery, to the left. The internal face of the tissue lies underneath the retina and receives incident light.

MATERIALS AND METHODS

Eyes were obtained from rough dogs, *Scyliorhinus canicula* (L.), smooth hounds, *Mustelus asterias* Cloquet, spur dogs, *Squalus acanthias* L., stingarees, *Dasyatis sabina* (Lesueur) and Texas skates or roundels *Raia texana* Chandler.

Spur dog eyes were dark-adapted. After the eyes were opened, they were left in selachian Ringer for a brief time, cut into segments and the retina was stripped off, leaving a preparation consisting of sclera, chorioid plus tapetum lucidum, choriocapillaris and pigment epithelium.

Material was fixed in 5% glutaraldehyde in selachian Ringer, distilled water or 0.1 M sucrose at 4 C. Fixative solutions were buffered to pH 7.4 with sodium cacodylate. The glutaraldehyde was washed out by repeated changes of cold 0.1 M sucrose and the material was refixed in 1% osmium tetroxide in distilled water (also buffered to a pH of 7.4 with sodium cacodylate). The tissue was dehydrated in ethanol embedded in araldite. Sections were cut on a Cambridge Huxley ultramicrotome at 0.5 μ for light microscopy and as thin as possible for electron microscopy. They were examined and photographed with a Philips EM 200 electron microscope, with an accelerating voltage of 40 kV. Considerable difficulty was experienced in cutting very thin sections, probably owing to the shattering of the guanine crystals. The butyl-methacrylate-styrene embedding medium of Kushida (1961), as modified by the Philips laboratory, was then tried. Blocks made from this proved easier to cut than the araldite ones. Sections were stained by vanadyl sulfate, vanadatomolybdate, uranyl acetate, or lead, following the technique of Millonig (1961). Staining was satisfactory with all solutions, little difference being observed between them.

RESULTS

APPEARANCE OF THE REFLECTING CELLS

When histological sections of the eye, which have been cut transversely, are examined with the light microscope, the reflecting cells of the tapetum lucidum, cut across, appear as thin bands; each has a nucleus part way along its length. The cytoplasm has very poor staining affinity; it exhibits fine lamellations approaching the limit of resolution of the light-microscope. The cells appear thin and shrunken after the reflecting material has been removed; thicker, when the reflecting material remains.

Upon examination of a fresh tapetum lucidum, stripped of retina, normal to its surface, one sees that the shiny surface is divided into irregular elongated areas (ellipsoids) with shadowy outlines. The appearance and orientation of the tapetal ellipsoids in different parts of the eye are well illustrated in Plate I of a paper by Denton and Nicol (1965c). In surface view one is looking through the largely transparent pigment epithelium (which is devoid of pigment) and the choriocapillaris with its contained blood corpuscles. Plate II of the same paper shows the pigment processes caught in the act of migrating over the exposed surfaces of the reflecting cells.

The ellipsoids are the exposed inner surfaces of the reflecting cells, exposed because the cells overlap. Viewing the tapetum lucidum normal to the surface of the pigment epithelium, one is looking at that portion of a reflecting cell which juts beyond the one overlying it (Fig. 1).

Sketches of tapeta, in surface view, part way between the center of the eye and the periphery, are presented in Figure 2. These illustrations reveal, clearly enough, that the internal margins of the reflecting cells are somewhat rounded, and that they overlap rather like fish scales, but with less geometrical precision and regularity. In the central fundus the ellipsoids are much more irregular (Fig.

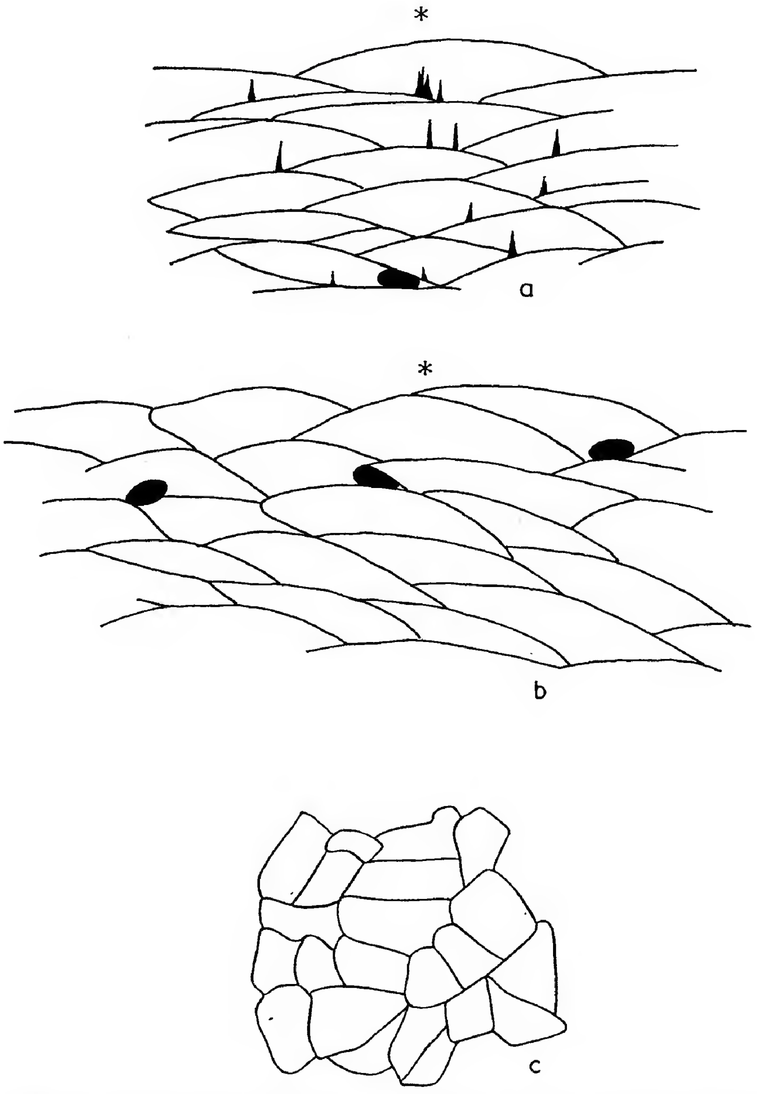


Fig. 2. Sketches of surface views of three tapeta lucida to show the form and arrangement of the ellipsoids. a, b are of regions of the eye part way between the center and the periphery. Some emerging pigment processses are shown in the upper sketch. Large black spots are loci of blood vessels penetrating the reflecting layer. From dark adapted eyes of spur dogs. *, direction of central fundus. ×400. c is of the central region of the tapetum lucidum of the Texas skate. ×250.

2), in contrast to the concentric repetitive patterning which they exhibit further peripherally. In the central fundus, also, the reflecting cells are stacked in piles, the inner cells of which extend past one another to form the irregular surfaces of the ellipsoids.

The ellipsoidal areas are over 100 μ long (for size range see Denton and Nicol 1964; 1965c). Now, in histologic sections, the dimensions of the reflecting cells, in profile, measured from the external limit of the tapetum lucidum to the choriocapillaris, range from about 40 μ to 60 μ. From these considerations it appears that the ratio of length to width is about 2 or 3 to 1 (Fig. 3).

Viewed as a whole, the reflecting cells are oriented, therefore, with their long axes aligned concentrically about the center of the fundus, this also being the alignment of the long axes of the ellipsoids. About half the reflecting surface of the cell is seen when it is viewed vertically to the surface of the pigment epithelium and less when viewed normal to the cell surfaces; *i.e.*, the amount of surface visible depends upon the angle of view.

It has been shown previously (Denton and Nicol 1965c), by direct measurements of reflection of light, that the reflecting plates of the tapetum lucidum are inclined around their long axes so that they face more towards the pupil than the plane of the tissue on which the plates lie, at least away from the center of the eye (Fig. 4). The reflecting plates, so measured, are the inner portions of the reflecting cells; and reflection depends upon the crystals which the cells contain. How are the crystals arranged within the reflecting cells, and what is the relationship between the orientation of the reflecting crystals and that of the reflecting cells?

ORIENTATION OF REFLECTING CRYSTALS

Crystals obtained from the tapetal reflecting cells are hexagonal in shape, resembling those occurring in the oriented silvery layers of the skin of silvery fishes.

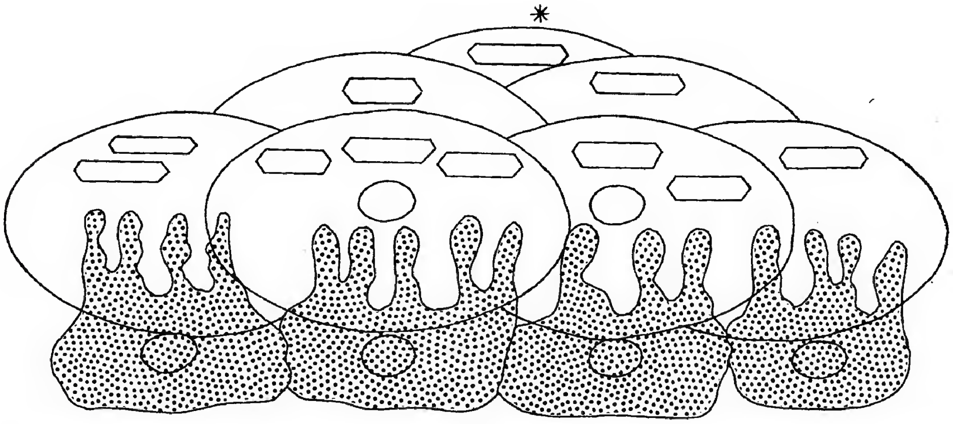


FIG. 3. Diagrammatic representation of the arrangement which the reflecting cells are thought to have. The internal surfaces of the reflecting cells are shown as they would be seen perpendicularly to their surfaces, which lie in the plane of the paper. The long axes (lengths) of the reflecting cells lie across the page; the short axes (widths), up and down the page. Melanophores are shown in stipple. The alignment of the reflecting plates (crystals) is shown (only a few representative crystals are illustrated). ×600 *ca.*

Dimensions of crystals are 5–10 μ wide by 14–33 μ long (Denton and Nicol 1965a). Descriptions and illustrations of these crystals are given in later sections.

By suitable arrangement of superior illumination it is possible to discern the reflecting platelets within the cells of fresh tapetal tissue. Hexagonal platelets of the same size and shape as those of crystals isolated from tapetal tissue are visible *in situ*. They are aligned lengthwise along the long axes of the ellipsoidal areas; therefore, along the long axes (lengths) of the reflecting cells (Fig. 3). They are staggered in overlapping rows so that they cover the entire exposed surface of the reflecting cell. The alignment is not perfect and there is a certain amount of irregular longitudinal deviation from side to side, just as there is in the alignment of platelets similarly arranged in scales of bleak, which are illustrated in Plate V of a paper by Denton and Nicol (1965a).

In the reflecting cells of the tapetum lucidum of selachians, the long axes of the platelets (and of the crystals) lie parallel to the surface of the tissue (inner surface of the eye) and, when the platelets (and crystals) are inclined to the surface of the tissue, they are inclined along their short axes (widths) and around their long axes (lengths).

COLORATION AND SPECTRUM REFLECTION

Spectrum curves for reflectivity across the visible spectrum have been published

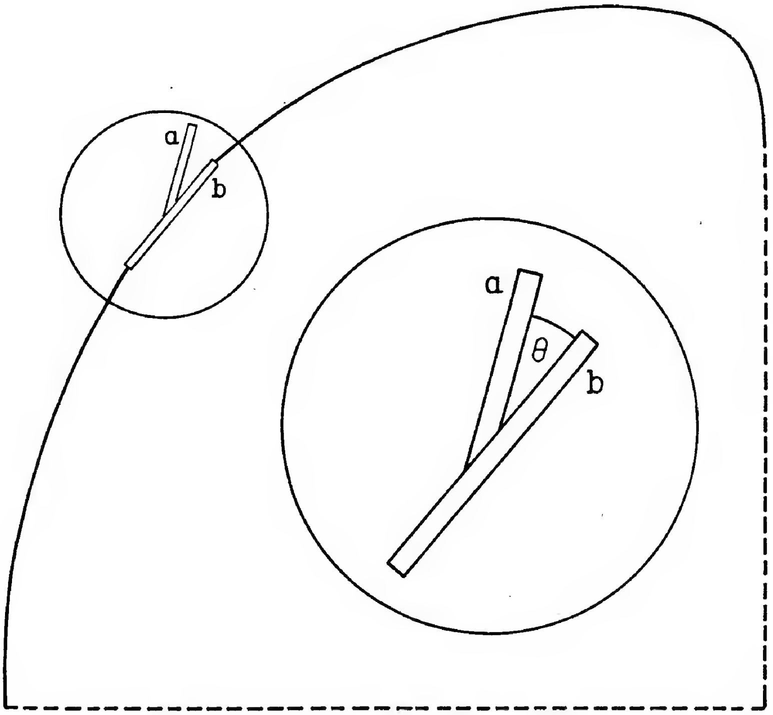


Fig. 4. Transverse section through half of an eye. The plane of the section is perpendicular to the surface of the tissue and passes through the central fundus. One reflecting plate, a, is shown, making angle θ with the surface of the tissue b. The angle θ increases from the center of the eye to the periphery. a is usually perpendicular to the direction of incident light.

for tapeta lucida of several cartilaginous fishes, *Scyliorhinus canicula, Deania calcea* and *Squalus acanthias* (Denton and Nicol 1964; Nicol 1965). These curves were obtained by means of an apparatus for measuring fractional reflectivity, using transparent filters of fairly wide band width. Reflectivity of the tapetum lucidum is high, over 80% and approaching 90% at wavelengths of maximal reflectivity. Spectrum curves for reflection of the tapeta lucida of *Scyliorhinus canicula* and *Squalus acanthias*, obtained with the use of a spectrophotometer, are presented in Figure 5. Measurements were made with a Unicam diffuse reflectance attachment (SP 540), against a block of magnesium carbonate as standard. The highest reflection occurs in the blue and green regions of the spectrum. The white tapetum lucidum of *Scyliorhinus canicula* reflects a high degree of light at all wavelengths (over 65% of blue and red light). The more bluish tapetum lucidum of *Squalus acanthias* reflects less effectively short and long wavelengths (reflection of violet and red light is less than 50%).

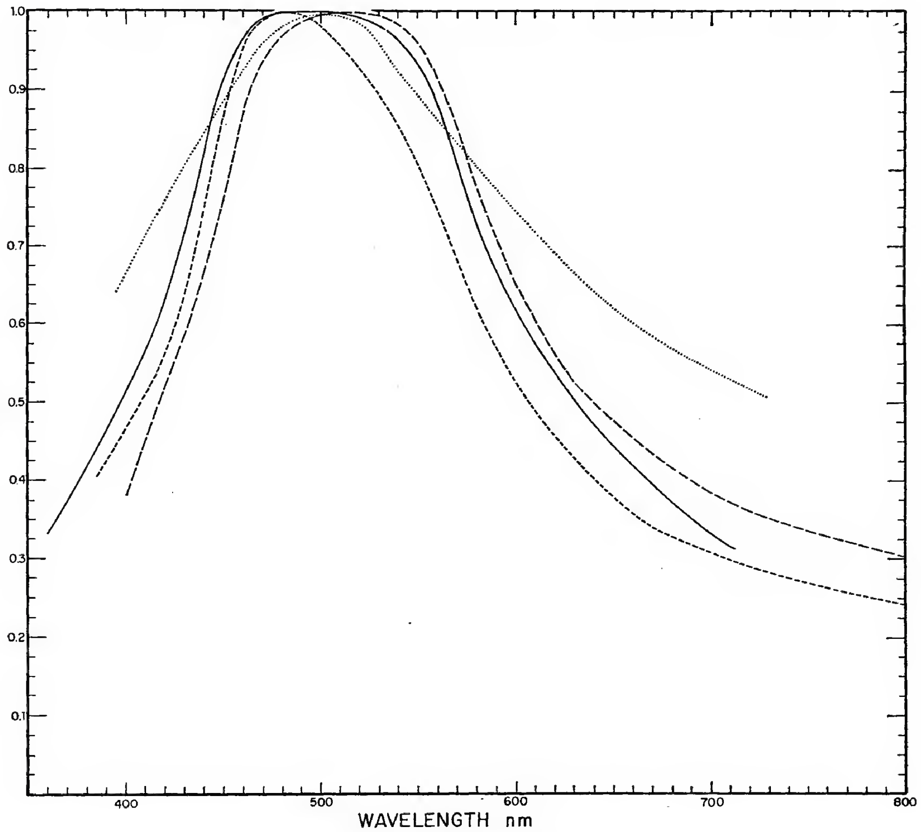


FIG. 5. Spectrum reflection of the tapetum lucidum of the rough dog (dotted line) and of three tapeta lucida of spur dogs (remaining lines). All curves normalized at λ_{max}. The instrument employed for making these measurements is designed to measure diffuse reflection, whereas that of the tapetum lucidum is specular. Because of these factors and the geometry of the apparatus, precise values of reflectivity at each wavelength were not obtained. The curves do show relative spectrum reflection.

A stripped tapetum of *Scyliorhinus canicula* was examined with a Zeiss pupillary spectroscope inserted in the tube of the microscope. A 2/3rd objective (N.A. 0.25) was used. The spectrum was continuous from blue to red (*ca* 430 nm to 670 nm), and the limits were the same as those of the comparison spectrum.

The overall appearance of the tapetum lucidum of *S. canicula* in macroscopic view is lustrous white or silvery. Microscopically, the ellipsoids which make up the surface of the tapetum are marked with small irregular brightly colored areas; yellow, green, red and blue. The platelets which can be discerned within the ellipsoids exhibit irregular markings and patterns of interference colors, or black bands, which mark to some extent the outlines of the individual crystals.

The predominant overall color of the stripped tapetum lucidum of the Texas skate *Raia texana* is blue-green. Adjacent ellipsoids in the same plane are colored blue and yellow-green, but microscopically there is recognizably a medley of colors present because yellows and reds as well show through in fine lines and bands.

By the use of vertical illumination one can distinguish individual crystals or their outlines, and these crystals are yellow, green, blue-green, blue, rose or pink. There are usually several colors on one crystal, as well as longitudinal streaks or bands of color, say green lines with intervening bands of rose, or longitudinal streaks of yellow, blue or green. Where purple occurs, it seems to be produced by longitudinal bands of blue and red. In some ellipsoids the crystals are predominantly green, in others blue, and in others they present a whole medley of colors; reds, blues and greens.

FINE STRUCTURE OF THE TAPETUM LUCIDUM

Sections for light microscopy and electron microscopy were cut vertically and longitudinally through the tapetum lucidum (Fig. 6).

Squalus acanthias.—Reflecting cells in electron micrographs range in thickness from 2.8 μ to 14.9 μ. The mean of 11 measurements is 6.6 μ. The cells become narrower towards their inner ends, where they are rounded or tapering.

The nucleus of the reflecting cell is narrow and elongated, 2 to 3 μ thick and about 15 μ long. It probably has the shape of a flattened disc; its membrane is sometimes folded inwards. It is located on one side of the reflecting cell, some distance along the length of the reflecting cell from the external and internal poles (Plate I, Fig. 1).

A large amount of the volume of the cytoplasm of the reflecting cells is occupied by conspicuous inclusions. These are elongated sacs, distributed along the length of the cell and closely stacked, one above the other, in tiers that occupy the thickness of the cell. The membranes of contiguous sacs lying above each other often close together and frequently follow each other's contours (Plate I, Fig. 2). The membrane is single and smooth. The axes of consecutive sacs often lie in slightly different planes; *i.e.*, when one sac ends another begins slightly above it or below it, frequently some distance away. Sacs above and below each other may overlap, and considerable gaps are often present in the cytoplasm where sacs are absent. In sum, there is no regularity in the disposition of the sacs, save that they are fairly closely spaced and the axes lie in planes generally parallel to each other and to the surface of the cell. Apart from the central axis, the material inside the sac has about the same density as the cytoplasm and exhibits no prominent features (Plate I, Fig. 2).

The sacs range in thickness from 124 to 497 nm; the mean thickness is 274 nm. The space between the sacs varies from less than 20 nm to as much as 5.5 μ; in places the membranes are almost touching. The mean interval between the membranes of adjacent sacs is 60 nm (Plate I, Fig. 2).

Measured from center to center the sacs are spaced at intervals ranging from about 150 to 630 nm, and generally from 275 to 400 nm.

Average values are misleading because they conceal the variation which is important for an explanation of the divergent interference patterns actually observed. For example, some 4 to 6 regularly disposed sacs at intervals between 350 and 420 nm may be interrupted by a gap or gaps which are often multiples of (2 to 5 times) these values, followed by another series of sacs at intervals of about 300 to 400 nm and so on (Plate II, Fig. 1).

Many of the sacs are split longitudinally, exhibiting empty spaces or clefts, the embedding araldite or methacrylate being missing. The clefts sometimes extend the entire length of the sac, or they are interrupted at irregular intervals. The

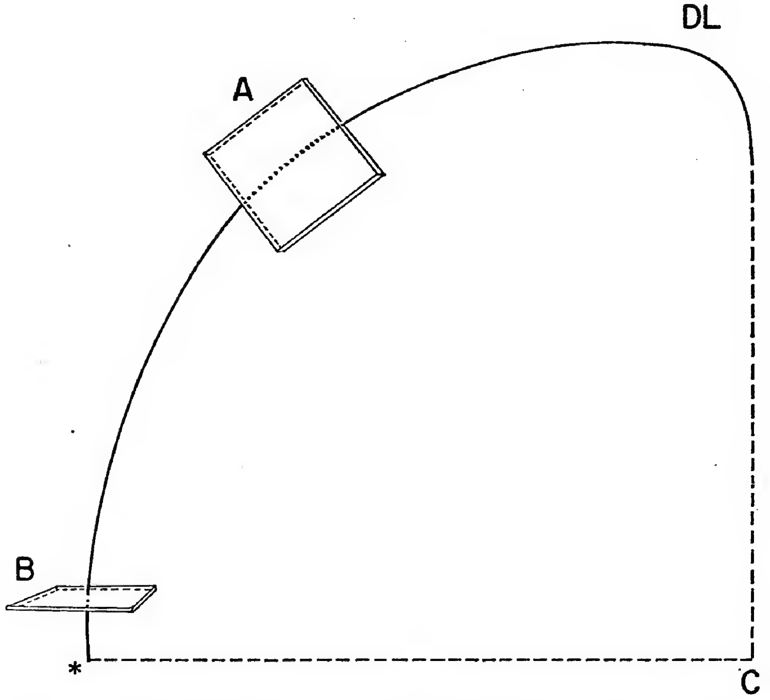


FIG. 6. Orientation of histologic sections cut perpendicularly to the surface of the tapetum lucidum. The diagram shows a section through half an eye in a plane perpendicular to the tapetal surface and passing through the central fundus. DL, dorsal or lateral margin of the oculus. C, center of the pupil. A, histologic section in a plane passing through the central fundus. B, histologic section in a plane at right angles to the plane passing through the central fundus.

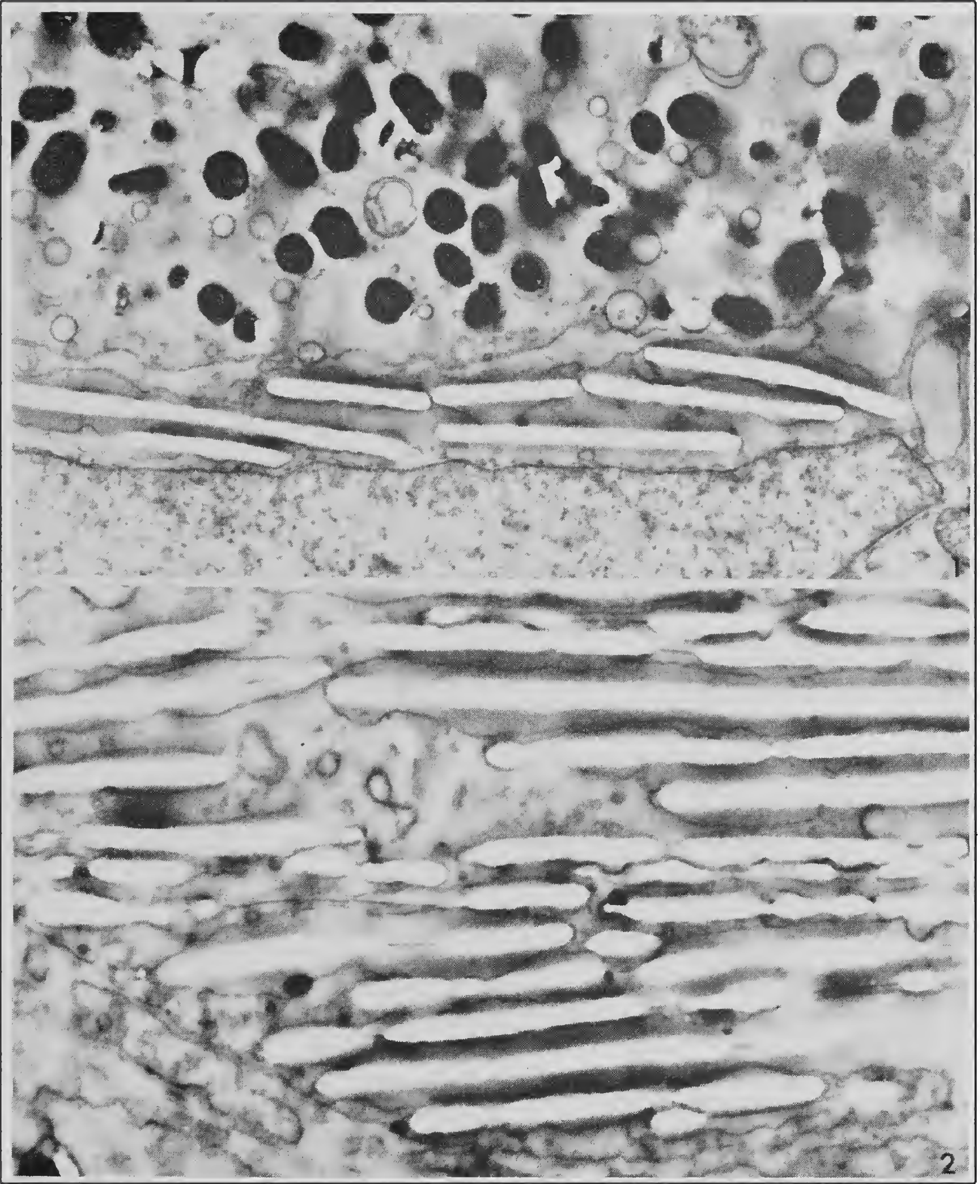


PLATE I. Electron micrographs of sections through the tapetum lucidum of *Squalus acanthias*. (Glutaraldehyde, osmium tetroxide, vanadatomolybdate.)

FIG. 1. Chorioidal pigment cell and reflecting cell lying adjacent to each other. The black masses in the upper cell are melanin granules. There is a little connective tissue in the space between the two cells. The elongated clear (white) areas are clefts or gaps, from which the crystals have been lost; they are enveloped by a membrane (the unit membrane of the crystal sac). There is a mitochondrion in the lower right hand corner. ×14,500.

FIG. 2. Section through the margin of a reflecting cell. In the cytoplasm there are vesicles and tubules of the smooth endoplasmic reticulum (SER). Axial lines are in evidence in some of the crystal sacs. ×24,700.

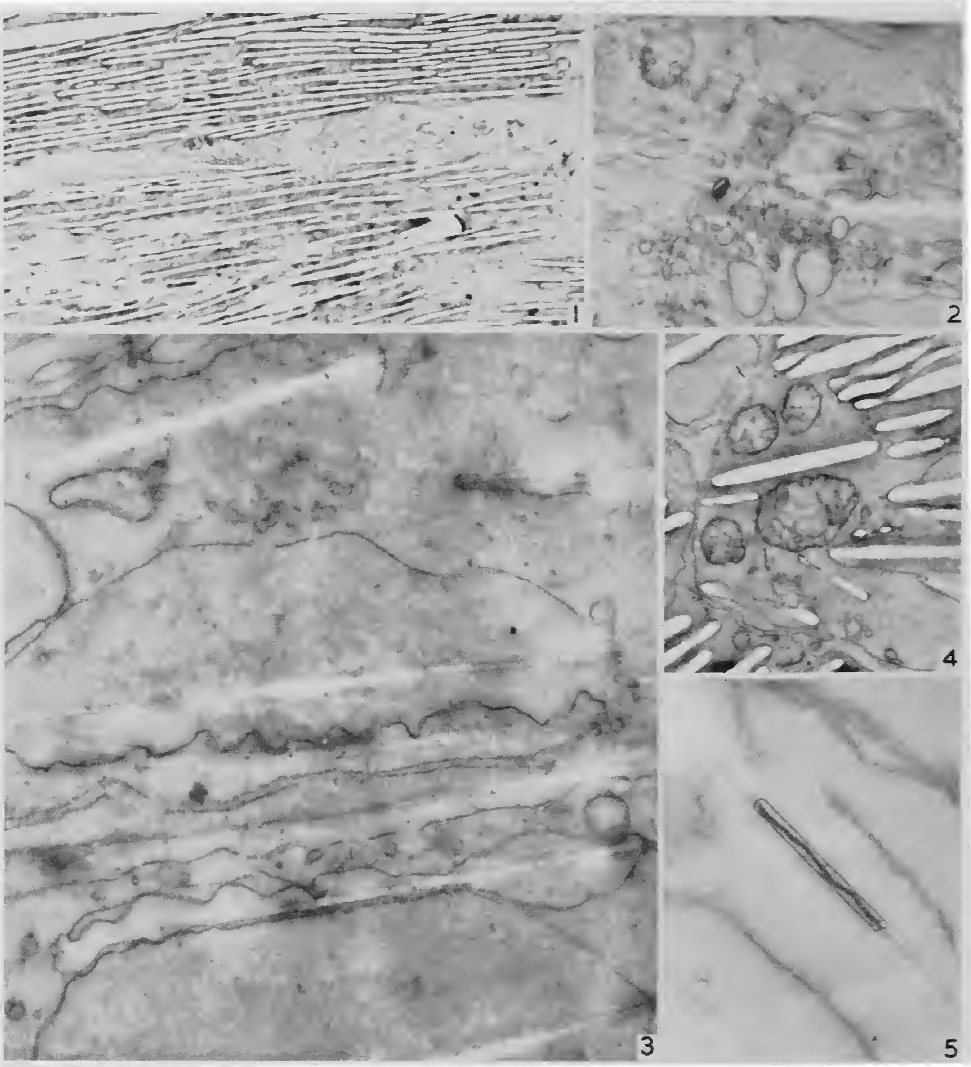


PLATE II. Electron micrographs of sections through the reflecting cells of *S. acanthias.*

FIG. 1. Three adjacent reflecting cells. ×7,250.

FIG. 2. Crystal sacs, mitochondrion, Golgi body, and vesicles or tubules of the SER. ×27,250.

FIG. 3. Crystal sacs and axial bands. ×46,325.

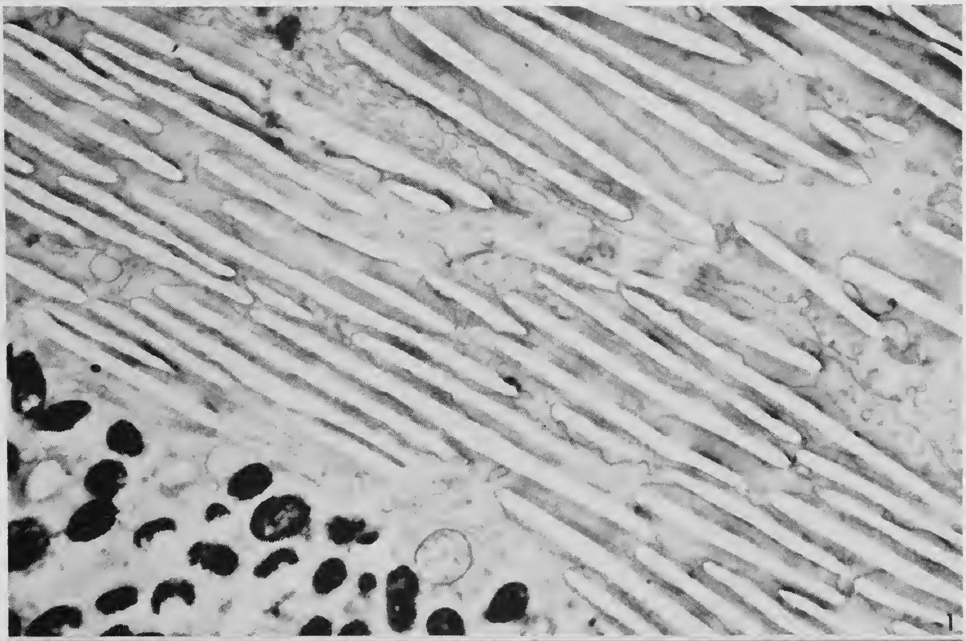
FIG. 4. Mitochondria near cell margin. ×32,250.

FIG. 5. Membranes of crystal sacs, axial lines and a crystal-like structure along an axial line. ×97,250.

PLATE III.

FIG. 1. Electron micrograph of a section through the tapetum lucidum of *S. acanthias.* Two contiguous reflecting cells above, chorioid pigment cell below. Crystal sacs in evidence. ×16,000.

FIGS. 2 and 3. Thin sections (1μ) of the tapetum lucidum of *S. acanthias*, photographed by dark-ground illumination. Fig. 2 shows shattered crystals; Fig. 3 shows intact crystals. A blood vessel penetrates the center of the section (the dark area) in Fig. 3. ×830.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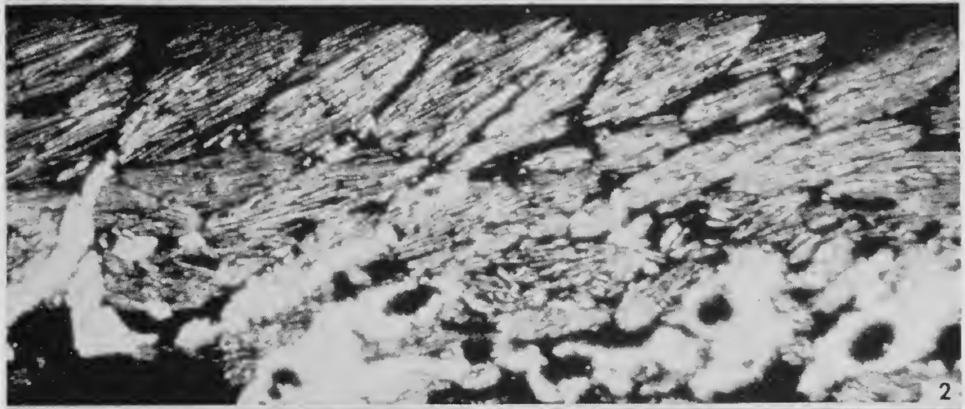
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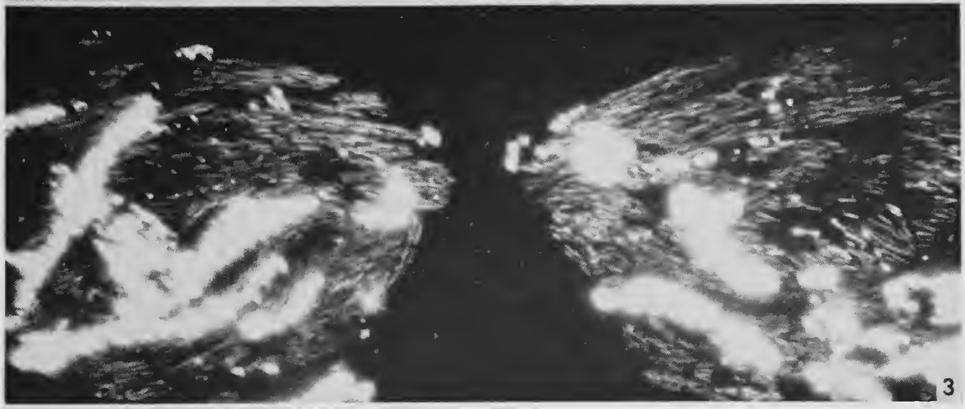
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splitting may be due to the brittle crystal fragments falling out during sectioning. The separation causes ugly gaps which do not, however, affect the relationships and appearance of the surrounding regions (Plate II, Fig. 1; Plate III, Fig. 1). The gaps widen in the electron beam. Presumably, there is poor adhesion between the araldite and the material occupying the axis of the sac.

After fixation with glutaraldehyde alone, and with glutaraldehyde followed by osmium tetroxide, the exposed face of the tapetum lucidum shines brightly in the methacrylate and araldite blocks. This appearance demonstrates that the crystals are retained in the tissue up to this stage of the histologic procedure. Sections were cut at 0.5 μ and at 1 μ from these blocks and were stained with toluidine blue for examination by light microscopy. With dark-ground illumination it can be seen that crystals are still present in these sections (Plate III, Figs. 2 and 3). The sections for electron microscopy were much thinner (grey and about 60 nm), and were picked up on Formvar, carbon stabilized films. Examined in the electron microscope with the lowest accelerating voltage that can be effectively employed for resolution, the sections show reflecting cells with gaps or clefts and entire reflecting cells with intact (*i.e.*, not split) crystal sacs; these can be seen side by side. The gaps in the reflecting cells widen in the electron beam when the supporting film is damaged and is free to move. The loss of the crystals from the very thin sections prepared for electron microscope examination happens either during the actual cutting or (perhaps more likely) in the expanding of the sections that follow. The breaking out of the crystals is less in methacrylate than in araldite, because the former makes a better bond with surfaces than does araldite.

Similar clefts are seen in electron micrographs of reflecting cells in the ventral photophores of *Maurolicus*. Bassot (1966) believes that the clefts result from the guanine platelets sublimating in the electron beam.

An axial line appears in many sacs that are not split, or between the clefts of sacs that are (Plate I, Fig. 2). The axial line lies more or less in the center of the sac.

Some sacs contain a pair of lines forming bands in the central axis (Plate II, Figs. 2 to 5). These range in width from 15 to 61 nm; mean width is 37 nm. A single short fragment of a band, standing out with great clarity is 40 nm wide. They may extend to the ends of the sacs, terminating in truncated faces or rounded tips.

Some sections contain a great deal of electron dense material, especially along the axes of the sacs. In the latter position the dense material is granular; appears as granules with coronae or as cross-hatched irregular broken bands. The cross-hatching gives the appearance of oblique bars. Adjacent bars may slope parallel to one another in one lamella, or lie obliquely left to right and right to left within a single lamella. Bands range in thickness from 25 to 59 nm; the mean is 37 nm. Conditions producing the irregular staining with heavy metals in these sections are not known.

In sections examined by electron microscopy the number of sacs disposed one above the other, across the short axis of the cell, varies from 9 to 18; the mean number is 12 (Plate II, Fig. 1; Plate III, Fig. 1). Sacs are reduced in number towards the ends of the reflecting cells (2 to 10).

Sections in araldite were cut at 0.5 μ, they were stained with toluidine blue and examined by dark-ground illumination. The reflecting cells exhibit rows of bright lamellae (Plate III, Figs. 2 and 3). The number of these lamellae, disposed perpendicularly to the short axis of the cell, varies from 7 to 20; the mean number is 12.

In some sections, examined by dark-ground illumination, individual lamellae are continuous (Plate III, Fig. 3). Many are slightly curved or bowed. In others, the lamellae are discontinuous, each one appearing as a row of beads or shining spots, rather as if the lamellae were shattered (Plate III, Fig. 2).

Further work is in progress, or planned, to investigate other cytoplasmic features of the reflecting calls of *Squalus* and other selachians. Free ribosomes are sparse. There is no rough endoplasmic reticulum. A smooth endoplasmic reticulum consisting of vesicles or convoluted tubules is well developed in the cytoplasm between and around the sacs. A small Golgi apparatus consists of a dense aggregation of cisternae and vesicles interconnected and opening into large vesicles. Mitochondria occurring at the periphery of the cell contain sparse cristae (Plate II, Figs. 1 to 4).

There is a small amount of connective tissue between the reflecting cells and other kinds of cells. The walls of the chorioidal tapetal pigment cells and the reflecting cells are often in close juxtaposition (Plate III, Fig. 1). Pigment particles in the pigment cells range from 250 to 800 nm in diameter.

A diagrammatic representation of the arrangement of elements in a reflecting cell is shown in Figure 7. The stereogram of Figure 8 attempts to show the organization of the tapetum lucidum and tissues immediately internal to it.

Scyliorhinus canicula.—The reflecting cells of *Scyliorhinus*, like those of *Squalus*, exhibit a grand development of laminated cisternae or "crystal sacs" which occupy a great part of the cytoplasm (Plate IV, Fig. 1). The cells are flattened discs having thicknesses of about 5 μ to 14 μ. The outer ends in the chorioid are rounded or tapering; the inner ends abutting against the choriocapillaris are somewhat rounded or abruptly flattened. Adjacent reflecting cells are in very close contact, the opposing plasma membranes approaching to within 18 to 50 nm of each other. The outer ends of the cells are also closely associated with the pigment cells of the chorioid. Away from the central fundus, towards the periphery of the eye where the reflecting cells lie obliquely to the inner surface of the eye, the processes of the pigment cells extend some distance inwards between the reflecting cells (Plate IV, Fig. 1).

The plasma membrane of the pigment cell closely follows that of the reflecting cell; the space between them when they are most closely associated is 10 to 30 nm. Occasionally there are large gaps between these cells, the space being filled with connective tissue fibers. The inner faces of the reflecting cells are separated from the endothelial cells of the choriocapillaris by a wide and dense layer of connective tissue fibers.

The nucleus of the reflecting cell is flattened, long and thin, and lies part way along the length of the cell near the plasma membrane. Long flat sacs or cisternae (the "crystal sacs") are abundant, occupying a great deal of the cytoplasm (Plate IV, Fig. 2). They lie in tiers, stacked above each other. There are 7 to 13 sacs (mean 11 sacs) across the narrow axis (thickness) of the cell, facing the inner surface of the tissue. The sacs are up to 40 μ long in sections and do not extend the entire length of the cell. They lie more or less parallel to each other, and superposed sacs overlap each other and interdigitate; consequently, there is a variable number of superposed sacs, at any one level, across the cell. The sacs extend to near the inner and outer ends of the cells, but only a few come close to the plasma membrane. They are generally long and thin, have undulating outlines, and each sac lies more or less in one plane. The sacs range in thickness from

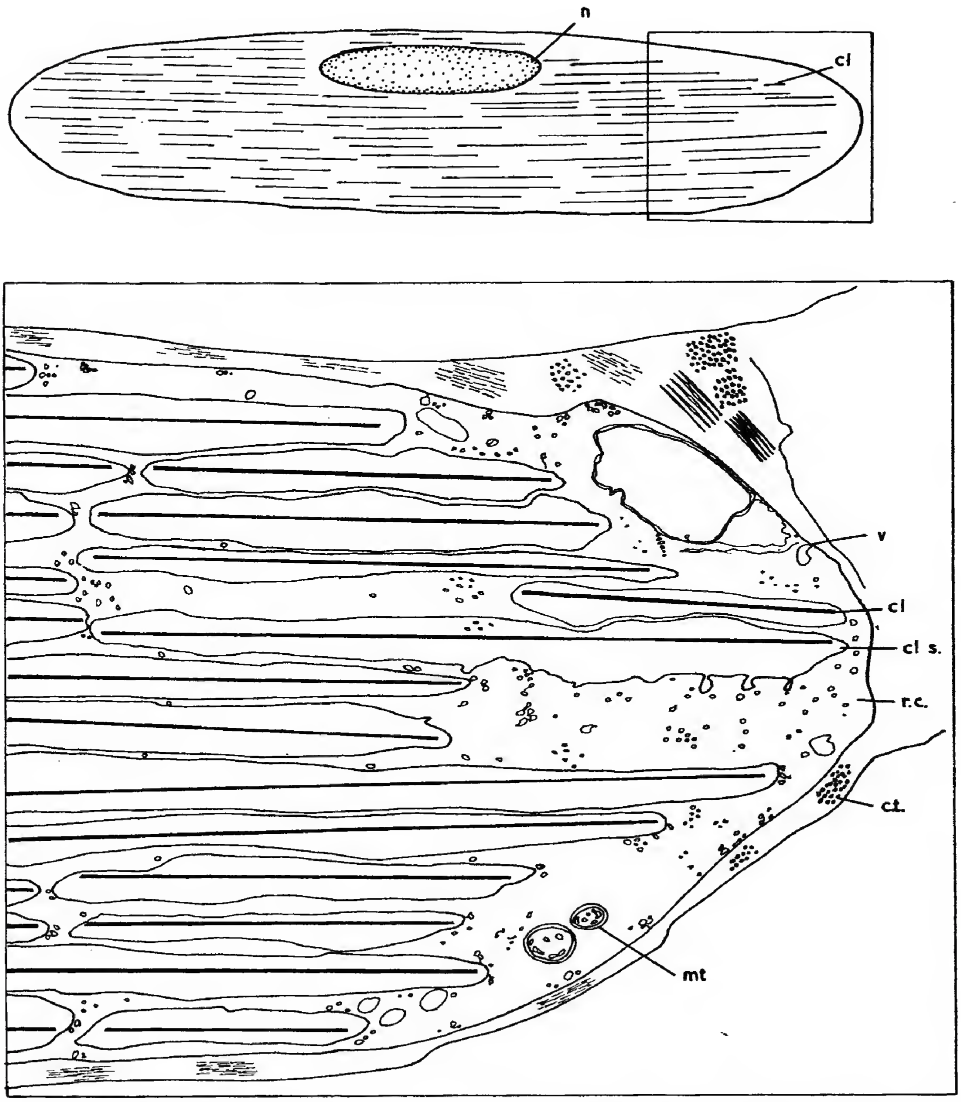


FIG. 7. Above, section across a reflecting cell. Axial planes of crystals shown. ×7,000 *ca.* Below, a section, enlarged, from the margin of a cell in the position indicated by the rectangle of the upper figure. ×30,000 *ca.* cl, crystal; cl.s., crystal sac; c.t., connective tissue; mt, mitochondrion; v, vesicle.

13 to 423 nm. They sometimes lie close together, the membranes of the sacs being 30 to 40 nm apart; at other places they are widely separated, by distances up to 1.4 μ towards the ends of the cells (Plate IV, Figs. 1 and 2). The intervals between the central axes (estimated) of adjacent sacs range from 198 to 3860 nm; the mean interval between central axes is about 300 mm.

Sacs are bounded by a smooth single membrane, which is irregularly undulatory (Plate IV, Fig. 2). The ground substance within the sac has about the same density as the hyaloplasm outside.

The sacs contain axial lines or bands, one running through the approximate center of the sac from side to side; they are missing, of course, when clefts occur. The lines or bands have fairly regular courses, but are not perfectly rectilinear. Sometimes they curve slightly and progressively or have slight kinks. The bands vary much in thickness along their lengths, from 15 to 50 nm. At the edge of each sac there is a small dense spot, about 30 to 40 nm in size, which is deeply stained. In some places the spot seems to touch the membrane of the sac; in others, to be separated from it by a small interval. When most distinct, the spots look like small black caps over the ends of the axial bands (Plate IV, Fig. 2).

The cytoplasm between the sacs contains abundant vesicles and tubules of smooth endoplasmic reticulum, and large vesicles, especially towards the ends of

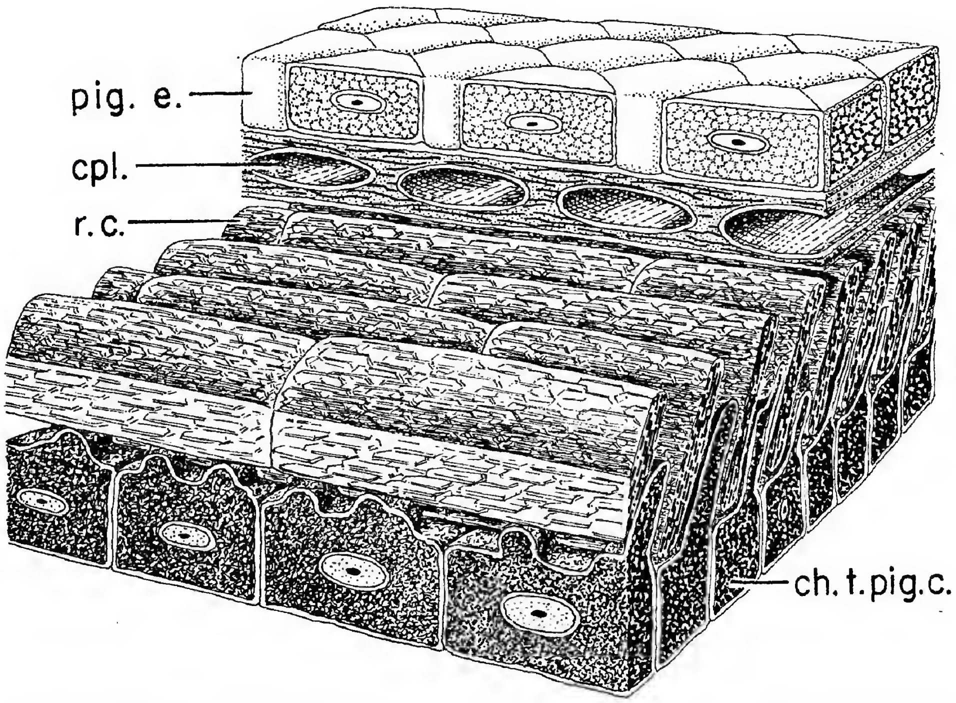
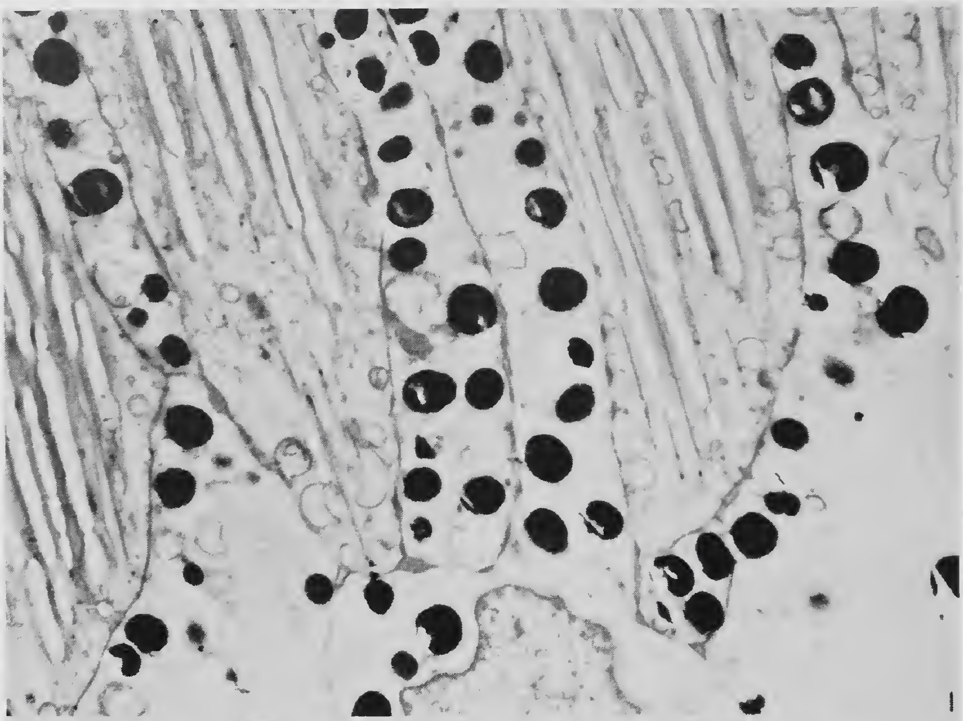


FIG. 8. Organization of the tapetum lucidum of the spur dog. The stereogram is of a block of tissue, part way between the center of the fundus and the periphery of the retina, where the reflecting cells slope with reference to the surface of the tissue. The top of the block faces the retina and this surface receives incident light; the reflecting platelets slope towards the central fundus. ×500 *ca. vide* Fig. 1.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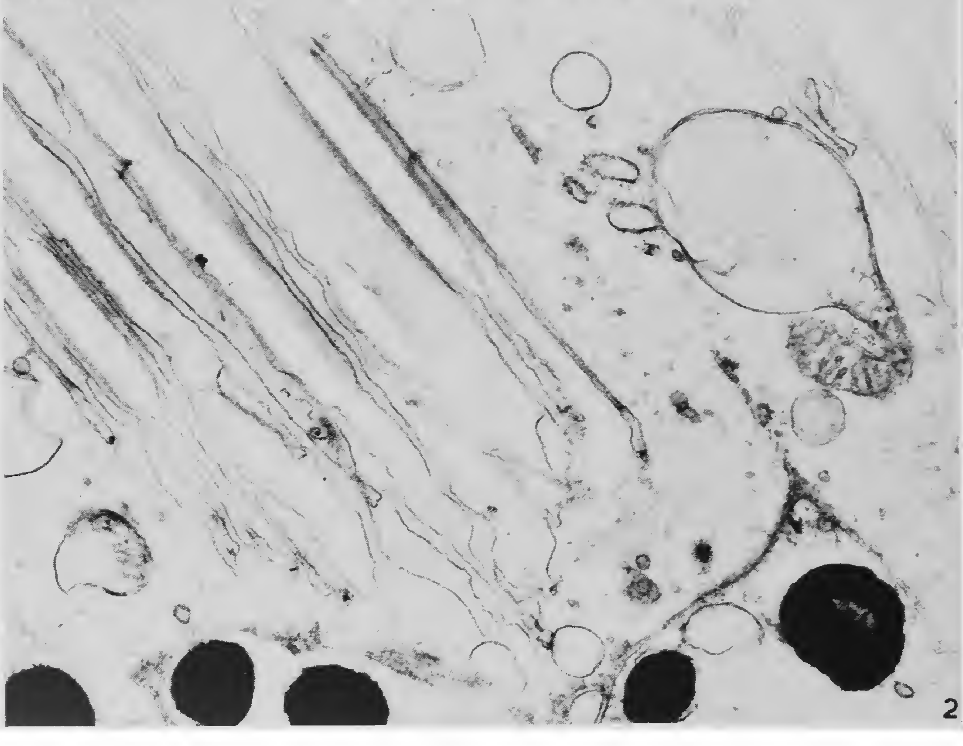
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the reflecting cell. The vesicles contain a good deal of wispy filamentous material. Free ribosomes are sparse, there is no rough endoplasmic reticulum. Mitochondria towards the ends or sides of the cell contain cristae, and the Golgi body towards the periphery consists of stacked cisternae, small vesicles and vacuoles (Plate IV, Figs. 1 and 2). The study of the intracellular components of the reflecting cells is being continued.

Adjacent chorioidal pigment cells contain large pigment granules, about 1 μ in diameter. They are sometimes broken during sectioning and must be very hard. The pigment granule is surrounded by a slightly ruffled unit membrane. The pigment cell contains vesicles and larger vacuoles, and mitochondria with sparse cristae. Pigment cells lie closely adjacent to each other. In places, the membranes are 20 nm apart; at others, they are separated by a layer of connective tissue fibers. One or two pigment cells or processes are associated with each reflector cell.

CRYSTALS

Reflecting crystals were obtained by gently scratching the surface of a fresh tapetum into a drop of distilled water on a glass slide, which was dried. Crystals collected in distilled water were also mounted on Formvar films on copper grids for examination by the electron microscope.

Crystals from the eye of *Squalus acanthias* were examined with the light microscope, using vertical illumination and a metallurgical objective. A range of crystals is shown in Plate V, Figures 1 and 2. They are hexagonal plates, varying greatly in size. Ratios of breadth to length lie between 1 to 1.1 and 1 to 8; the mean is around 1 to 3 and 1 to 4.

The crystals are certainly exceedingly thin because, when spinning freely in the water, they momently disappear when they turn and are being viewed edgeways; *i.e.*, perpendicularly to their flat surfaces. The flat surfaces have dimensions in the range: breadth, 0.72 μ to 8.4 μ; length 2.6 μ to 24.2 μ.

Electron micrographs of isolated reflecting crystals are shown in Plate V, Figures 2 and 5. Shapes range from long and narrow to short and squat. A feature of particular interest is the frayed edges found at the ends of crystals (see especially Plate V, Fig. 3). The long sides, on the other hand, generally have smooth edges (Plate V, Fig. 3).

Initially, isolated crystals give an electron diffraction pattern, indicating the existence of a regular crystalline lattice. Soon after exposure to the electron beam the diffraction pattern disappears. The shape and general appearance of what was the crystal, as seen by the electron microscope, remain completely unaltered. We do not know that the crystal is a homogeneous body. Assuming that it is (and this is the simplest hypothesis), we may believe that the energy of the electron beam

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PLATE IV. Electron micrographs of sections through the tapetum lucidum of *Scyliorhinus canicula*; from the periphery of the oculus.

FIG. 1. Several reflecting cells abutting against the pigment cells of the chorioid. ×12,200.

FIG. 2. Outer end of a reflecting cell. Crystal sacs, axial bands, mitochondria and SER are seen. ×33,600.

destroys the crystalline structure, but leaves an amorphous mass retaining the shape and configuration of the pre-existent crystal. Crystals obtained from tapeta lucida of other selachians are similar in size and shape to those of *Squalus acanthias.* They are nearly always hexagonal plates, several times longer than wide.

The crystals obtained from *S. acanthias* and *Dasyatis sabina* are mostly intact.

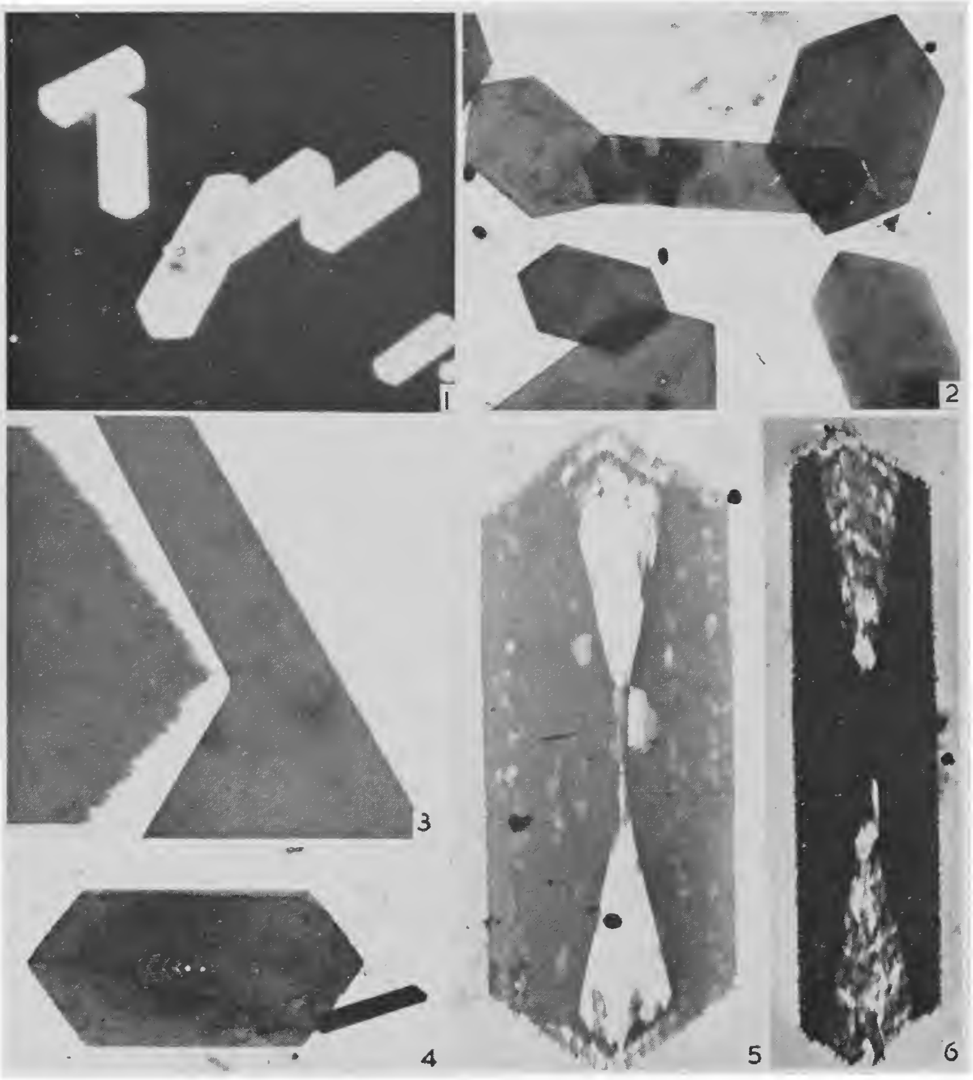

PLATE V. Crystals from tapeta lucida.

FIG. 1. Crystals from *Squalus acanthias*, photographed by vertical illumination. ×600.

FIG. 2 to 6. Electron micrographs of crystals of *Scyliorhinus canicula.* Fig. 2, an array of crystals. ×3,500. Fig. 3. Left, end of a crystal. Right, side of a crystal. ×32,250. Fig. 4. Disparity in size and shape; chevrons on larger crystal. ×3,500. Figs. 5 and 6, patterns of disintegration. ×5,600.

But those obtained from *Scyliorhinus canicula* and *Raia texana* are often incomplete, showing irregular pits, chevrons, holes and marginal nicks (Plate V, Figs. 4, 5 and 6).

The thickness of the crystals of *S. canicula* was estimated from electron micrographs of crystals which had been shadow-cast with palladium-gold at an angle of 15°. In these micrographs the crystals range in thickness from 23 nm to 58 nm; the mean thickness is 38 nm.

COLORS, INTERFERENCE AND ESTIMATIONS OF CRYSTAL THICKNESSES

Isolated crystals are uniformly grey or colored in various ways. Single crystals are usually a homogeneous grey, a little brighter than the background. But single crystals, overlapping crystals and jumbled masses of crystals are often colored. Two types of coloration are seen.

One type is seen in single and in overlapping crystals. In this type, part of a crystal may be grey, part colored, or all of a crystal may be colored and marked with successive bands or concentric circles of various colors. Similar complex color patterns are seen in superposed crystals. These effects resemble Newton's rings and interference fringes, and they are produced seemingly by multiple reflection and interference between the lower surface of the crystal and the surface of the glass on which it lies, when the two surfaces are separated by an air space.

A second type of coloration occurs where two or more crystals overlap. The colors coincide strictly with the outlines of the crystals; where two overlap, all the region of overlap is orange or purple; where three overlap, all the region of overlap is blue; and so on. One can thus distinguish the outlines of the individual crystals, even thought they are oriented in several directions and lie above one another, by their several colors.

Colors and tones of the second type are produced by interference between the rays of light reflected from the upper surface of the crystal; *i.e.*, the crystal-air interface, and those reflected from the lower surface of the crystal. Because the crystal has a higher refractive index than air, the light rays reflected from the upper surface of the crystal undergo a phase change of π radians upon reflection, the rays reflected from the lower surface do not; thus, there is a phase difference of $\lambda/2$ between the rays reflected from two surfaces. Constructive interference between the light waves occurs when the thickness, d, of the film is $\frac{m}{2n}\lambda$ and destructive interference when d is $\frac{(m\text{-}1/2)}{2n}\lambda$; where m is any integer, and n is the index of refraction of the film or lamella.

The tones and colors exhibited by the crystals of the tapetum lucidum belong to the first and second orders. The several interference colors exhibited by two or more superposed crystals provide a simple means of estimating the approximate thickness of the crystals, and of checking the estimates against each other. When the crystals are superposed and are producing interference colors of the first and second orders, it is assumed that they are in optical contact. Furthermore, it is assumed for these calculations that the refractive index of the crystals is about

1.85 (Denton and Nicol 1964; 1965b). The results are presented in Table 1. The estimated optical path lengths are given, enabling the path length to be recalculated should a mean precise value for the refractive index become available.

MEASUREMENTS OF CRYSTAL THICKNESS WITH AN INTERFERENCE MICROSCOPE

Measurements of the thicknesses of single crystals and of several superposed crystals were made with an AO-Baker Interference Microscope. A 40× shearing objective was employed; monochromatic green light was secured by interposing a 546 nm interference filter in the light path. The crystals, obtained by scratching the surface of pieces of tapeta lucida, were dried on glass slides. Preparations were made from tapeta lucida of stingarees, Texas skates and rough hounds. To measure the optical path, crystals were covered with distilled water and the matching method was used. Unfortunately, there is a good deal of subjectivity involved in these estimations because the point of extinction is difficult to determine and the rotation of the analyser is only a few degrees. Thicknesses of the crystals lie in the range 30 to 70 nm.

Measurements of the thicknesses of crystals secured from tapeta lucida indicate that those of different species have different thicknesses. Of the three species examined, the crystals increase in thickness in the following order: stingaree < rough hound < Texas skate. From the colors exhibited by superposed crystals it appears that crystal thicknesses in these species are in the relationship: stingaree < rough hound ≦ Texas skate.

TABLE 1

Interference colors of crystals, estimated optical paths and thicknesses

Animal	Number of crystals	Color	Optical path length xnd	Thickness d(*ca*)
Dasyatis sabina	1	grey silver	< 90–120	< 50–65
	2	gold	120–190	35–50
		orange-purple	190–210	50–60
		purple	210–260	60–70
	3	blue, blue-green	260–320	45–55
	4	green to orange	410–460	55–60
	5	blue	490–570	55–60
Scyliorhinus canicula	1	grey	< 90–120	< 50–65
	2	gold	120–180	35–50
		orange-red	180–205	50–60
		reddish-violet	205–215	60–65
	3	blue, blue-green	260–315	45–55
Raia texana	1	grey	< 90–120	< 50–60
	2	orange	175–205	50–60
		purple	205–260	60–70
	3	blue, green	290–315	50–55
	4	green	315–410	40–55
	5	purple	450–490	50–55

LEVELS OF GUANINE IN SKIN AND EYES

Skin and eyes were analysed for guanine by Hitching's method (Hitchings 1941). In this method the tissue is extracted with sulfuric acid; the solution is made slightly alkaline and the purines are precipitated with cupric sulfate. The precipitate is taken up in hydrochloric acid, the copper precipitated with hydrogen sulfide, and filtered off. Color is developed with Folin-Denis-Ciocalteau reagent, and absorption is determined photometrically. The procedure also detects xanthine, the color developed by guanine and xanthine being additive. Pirie and Simpson (1946) identified the crystalline material which they obtained from the chorioid of spur dogs as guanine; and Hitchings and Falco (1944) demonstrated the same for the silvery layer in the skin of the greenfish *Girella nigricans*. In the skin of the coho salmon there is some hypoxanthine and possibly guanylic acid and pterins, in addition to guanine (Markert and Vanstone 1966).

Two initial analyses were made on fish skin and eyes. White belly skin of *Microstomus kitt* contained 1.34 mg/g wet weight. Dogfish eyes (*Scyliorhinus canicula*) contained 2.20 mg guanine/g wet weight and 26.3 mg guanine/g dry weight.

A second series of analyses was made of skin and eyes of rough dogs (*Scyliorhinus canicula*). Five fish ranged in length from 42.7 to 62 cm, and weighed 296 to 767 g. Eyes were opened, fronts (cornea, iris, lens) cut out; vitreous and retina were removed. The tissue extracted was the wall of the eye, containing sclera, chorioid and pigment epithelium. The whole wall of the eye and circles 5 mm in diameter were analysed. In addition pieces of skin, 13 mm in diameter, were taken from the back and flanks. Results are given in Table 2.

Amounts of guanine were much higher in the tapetum lucidum than in the lower region of the eye where a tapetum lucidum is wanting. The amount of guanine in the upper wall of the eye is also much greater than in the skin. Since we are dealing with guanine in reflecting cells, quantities expressed in terms of surface area are more relevant than in terms of weight of tissue. The last column shows that a unit surface of tapetum lucidum chorioideale contains three to five times more guanine than black areas of chorioid and skin.

Values of guanine in fish skin, adduced by Sumner (1944), may be used for comparison. He held *Girella nigricans* (a teleost) on black and white back-

TABLE 2

Amount of guanine in skin and eyes of *Scyliorhinus canicula*

Tissue	Guanine Wet weight mg/g	 Dry weight mg/g	 Surface mg/cm²
Dorsal eye, 5 mm circle	23.3	123.0	0.74
Central eye	6.72	36.1	0.24
Eye, complete wall	2.89	24.17	. . .
Eye, do	3.40	23.00	. . .
Skin, flank, 13 mm circle	0.314	1.33	0.11
Skin, dorsum	0.484	2.72	0.17

grounds. On black backgrounds, the skin contained 3.68 mg guanine/g dry weight of skin; on white backgrounds, 14.78 mg/g dry weight.

DISCUSSION

Histological examination of the tapetum lucidum of selachians, by light microscopy and electron microscopy, has clarified certain features and aspects of the reflecting cells and simultaneously raised other problems. The discussion that follows pertains especially to the dogfishes *Squalus acanthias* and *Scyliorhinus canicula.* The tapeta lucida of other selachians may differ significantly in some respects from those described in the previous pages.

So far as we know, the chief function of the reflecting cells of the tapetum lucidum is to reflect incident light. This is accomplished by regularly arranged plane plates, which are crystals having the shape of elongated hexagons. The crystals lie within reflecting cells. Those of one cell are similarly oriented, the flat surfaces being parallel to each other and the long axes directed in the same direction. Also, the crystals are aligned with their long axes parallel to the long axes of the cells and to the surface of the tissue; they are disposed with these axes lying concentric to the center of the fundus. The flat surfaces of the crystals are parallel or almost parallel to the broad flat surface of the reflecting cell that projects beneath the choriocapillaris (Fig. 3). This arrangement means that when the reflecting platelet is tilted with reference to the surface of the tissue, as it is in all regions that do not lie at or near the center of the eye, the tilt is accomplished by a change of orientation of the reflecting cell as a whole. The crystals retain a constant orientation within the reflecting cell. The reflecting cells and the plane surfaces of the reflecting crystals lie approximately perpendicular to the light reaching them in all parts of the eye. A consequence of the arrangement is that the crystals, when tilted away from the retinal surface, are tilted along their long axes, which remain parallel to the surface of the tissue.

In the reflecting cell of the selachian tapetum lucidum, the dimensions of the cell are obviously such that the crystals could be aligned with their long axes along either the length or width of the cell. Similarly, even where the reflecting cells are steeply pitched to the surface of the tissue, as they are near the periphery of the eye, the depth of the tapetal layer and the width of the cells are still adequate to accommodate the crystals if they were arranged with their long axes along the width of the cells. Either way of tipping the crystals (along their short or their long axes) with reference to the surface of the tissue, would produce the controlled reflectivity observed.

Analogous conditions are encountered in the oriented reflecting layer of silvery teleosts, where the crystals are arranged also with their long axes parallel to the surface of the scale and, when they are tilted, they are tilted around the long axes. It has been suggested (Denton and Nicol 1965a) that this arrangement serves to accommodate the platelets, when oblique to the surface, in a limited space. Also, it produces a situation by which the long sides of the platelets can be used as reference lines which can be made parallel to the under surface of the scale; this effect ensures constancy in the angles of the platelets by which they reflect light coming to them from above or below the fish. There is an advantage to the arrangement of the crystals in the tapetum lucidum in this respect: there is more room in the long axis (length) of the cell for staggering and overlapping of crystals arranged lengthwise, thereby producing a more uniform reflecting pile with greatest number of reflecting interfaces, than there would be if the crystals were arranged with their long axes at right angles to the length of the cell. In such thin cells, to contain twelve or so reflecitng layers, the superposed crystals must lie parallel to the faces of the cell, and tilting must involve a change in orientation of the whole cell (or, alternatively, a change in cell thickness, which does not occur). It is a nice question, indeed, if the exact orientation of the reflecting platelets is a consequence rather of a precise arranging of prospective reflecting cells during ontogeny (and subsequent growth) rather than of a determinant binding the precise orientation of the crystals to the direct influence of the position of the surface of some contiguous tissue. In the former postulate, the disposition and alignment of the crystals would then be dependent on intracellular guide lines or cellular polarity; and the overall orientation of the developing reflecting cells is fixed with reference to surrounding tissues and surfaces. The problem then becomes one of spatial precision in cellular organization during morphogenesis. We may note, in this regard, that oriented cells in the reflecting layer of parr (young salmon) appear well formed and conspicious in sections of the upper flank; this is tissue that becomes silvery in the smolt stage. A study of the deposition of guanine in oriented reflecting cells and the organization of such cells in metamorphosing fish, such as juvenile salmon, would be of much interest.

Microscopic examination of the surface of fresh tapeta lucida shows only that the reflecting surface contains colored platelets of the size and shape of crystals that can be secured by rupturing the tissue. Several lines of evidence show that the platelets really are composed of stacks of superposed lamellae or crystals. Indications of stratification in reflecting cells are seen in sections examined with the light microscope, but such lamellae lie around the limit of resolution. Thin sections examined by dark-ground illumination contain many brightly shining lamellae, which lie parallel to the plane surfaces of the cell and to each other. There are, on the average, about twelve lamellae superposed and adjacent to each other in a section across the thickness of the cell. These lamellae are probably sections through crystals. In electron micrographs there are about twelve superposed sacs or bands similarly oriented; these are related to the crystals, and are indicative of their number and disposition.

Crystals (crystal sacs) seen in electron micrograph are enclosed in membranes. It seems that each sac contains a crystal which occupies its central plane. Presumably, the crystal is laid down and augmented inside the sac, which is the intracellular organelle controlling crystal formation. A sac is to be pictured as a large bolster-shaped organelle loosely enveloping a crystal, between the latter and the walls of the sac there is often a large interval. Sacs have been seen only in section. Presumably, they are elongate in the long axis of the reflecting cell, and they are superposed across the thickness of the cell.

The iridophore of teleosts is another kind of reflecting cell containing piles of reflecting platelets. These cells are shiny green or blue, they contain 12 or more superposed platelets, regularly aligned, but the physical parameters of the reflecting elements are rather different from those of the tapetum lucidum (Kawaguti and Kamishima 1966).

No exact analysis of clean crystals from the reflecting tissues of fishes appears to have been made. The belief that they are of guanine is based upon this correlate: that there is much guanine in silvery skins of fishes and in selachian chorioids; the levels of guanine in these two kinds of tissues are approximately the same (Table 2). There is a possibility of some organic core in the crystals.

In studies of the photophores of teleost fish, Bassot (1960; 1966) observed that sections of the reflecting layer (in electron microscope photographs) show platelets arranged with great regularity. From X-ray diffraction studies it was concluded that the reflector of the photophore of *Maurolicus* contains guanine (Barraud *et al.* 1959). Bassot (*loc. cit.*) was led to believe that the platelets seen under the electron microscope were made of guanine, which sublimated rapidly in the electron beam. A photomicrograph of the reflector of *Maurolicus* (Bassot *loc. cit.*) shows aligned clefts or gaps extraordinarily similar to those which we have found in the tapetum lucidum of the selachian eye, with the exception that the clefts are not as long. They are 0.07 μ to 0.2 μ thick, and 1 μ to 1.8 μ long in the reflector cells of *Maurolicus*. Moreover, in the cytoplasm around some of the gaps it is possible to see occasionally lines indicative of sac membranes.

In a short abstract, Bernstein and Dietrich (1960) make mention of crystalline plates, lying parallel to each other, in chorioidal cells of selachians, which were examined by electron microscopy.

Because reflection of light from the surface of the tapetum lucidum of dogfishes is very specular (Denton and Nicol 1964) it is to be expected that the reflecting laminae of crystals should have regular flat surfaces (*i.e.*, a high degree of planeness) and be arranged parallel to each other. We know that the reflecting crystals are flat and very thin. In fact, there is some spread of light from a preparation of stripped tapetum lucidum, amounting to 5° or 6° (Denton and Nicol 1964); part of this scatter may occur in the thin transparent tissue lying over the reflecting cells. Again, a fresh tapetum examined by superior illumination is generally bright, but the occasional ellipsoidal area is darker than the others, or has a different hue. Light-microscopy shows that there is some variation in the tilt of adjacent cells, as seen in section. The electron microscope reveals that there is a certain amount of variation in the alignment and disposition of the reflecting lamellae. These (although aligned generally parallel to each other and having their faces parallel to the surface of the cell) do deviate from regularity to some degree. The great range of spectral colors displayed by reflecting platelets in fresh tapeta lucida indicates that the irregularity necessary to produce the diversity of interference colors observed must exist also in the reflecting cell; either in variations in the angle which the cell makes to the surface of the tissue or which the crystals make to the cell surface, or in variations of crystal thickness or spacing, either singly or in conjunction.

The high degree of reflectivity and the manifold colors exhibited by the tapetum lucidum are produced by the constructive interference between light waves reflected from multiple thin plates, in particular, by superposed stacked crystals. This situation is very similar to that in the silvery scales of teleosts such as the bleak (Denton and Nicol 1965a). The general problem of interference colors and metallic reflections of biological materials, especially insect cuticles, insect scales, and feathers, has been analysed with great elegance by Lord Rayleigh (1923) and by Mason (1927a;b), and the literature has been reviewed by Fox (1953). The brilliant iridescent colors of the wings of beetles, for example, are produced by multiple thin films. In the integument there are some three to ten of these films which are separated from each other by laminae having a different refractive index. When the surface of the integument is smooth, it has a metallic iridescent appearance, and the reflection can be very specular. The predominant color depends on the spacing between the films and the thickness of the individual films. The colors seen are those of Newton's series of the second and third orders. The high degree of reflection is achieved by the summed reflections of multiple thin films. With increasing angle of incidence, there is a change of hue towards a color lower in the order. The reflected and the transmitted colors are complementary in hue and, in general, the transmitted colors are more saturated than the reflected colors. A dark background enhances the saturation and brilliancy of the reflected color, by absorbing the complementary transmitted colors (Mason 1927a;b).

Evidence for the thickness of crystals in tapeta lucida has been secured in several ways. Measurements of sectioned crystals in electron microscope photographs are not wholly reliable, because of the damage and distortion that the tissue experiences during preparation, sectioning and examination. Recognizable within the crystal sacs of the reflecting cells are axial bands ranging in thickness from 15 to 60 nm; the mean thickness of those of *Squalus acanthias* is 37 nm. Estimated thickness of shadow cast crystals of *Scyliorhinus canicula* range from 23 nm to 58 nm. The various interference colors which single and superposed crystals show in vertical illumination provide strong evidence that crystals from any one tapetum lucidum vary somewhat in thickness, and that they vary in thickness from species to species. The crystals of the roundel skate, for example, appear to be thinner than those of the stingaree. Estimates based upon interference colors predict crystal thicknesses of 30 to 70 nm; median around 50 nm (*vide* Table 1). Measurements of thickness made with an interference microscope adduce mean values of 60 to 70 nm for the crystals of rough hounds, Texas skates and stingarees.

The refractive indices of the reflecting platelets of fishes are not certainly known. Denton and Nicol (1965b), using an immersion method, give a range of 1.8 to 1.9 (median 1.85). But Ueda and Matsuzawa (1956) present other values; *viz.* $n^{20}{}_{D}$ 1.682–1.716 for hexagonal crystals, and 1.716–1.733 for raphidial crystals (of "guanine"). Some information about the birefringence of the crystals to relate to their refractive indices is desirable.

In a reflecting cell about 6μ thick, containing twelve superposed crystals equally spaced, the interlamellar distance, d, is about 0.4μ. In electron microscope photographs the spacing lies between 0.15 and 0.63μ, and generally between 0.3μ and 0.4μ.

Anderson and Richards (1942) have calculated the spectrum reflectance that would be given by a pile of twelve thin films spaced equidistantly at regular intervals from 0.2μ to 0.29μ apart (in air). They show that for such a perfect pile of films only a narrow band of wavelengths with maxima at $m\lambda = 2d_p$ is reflected (m is the order of reflection and d_p the spacing between adjacent films), and intensities for other wavelengths are low. Their figure 11 gives curves of intensity of reflectance against wavelength for piles of films at selected spacings. This simple theoretical case is identical in its implications with the situation in the reflecting cells of dogfishes, if the platelets are regarded as emitters of extreme thinness and allowance is made for the refractive index of the medium.

For a series of finite films the wavelength of maximal reflectance at normal incidence is:

$$m\lambda_{max} = 2d_p = (d_1n_1 + d_2n_2);$$

where d_1, is the spacing; d_2, the thickness of the film; and n_1, and n_2, the refractive indices of the media. It is a simple matter to calculate the wavelengths of maximal reflectance and the colors to be expected from such a system for different values of d_1 and d_2. When d_1 is 150 to 550 nm and d_2 is 20 to 50 nm, the reflectance progressively advances through first, second and third orders and the whole visual spectrum from 400 nm to 700 nm is spanned; at intervals the reflectance is outside the visible in the near ultra violet and the infra red. For example, for values of d_1 equal to 300, 350 and 400 nm, when d_2 is 37 nm, maximal reflectance (second order) lies at λ400, 540 and 607 nm, respectively.

In a system of this kind, as the angle of incidence decreases, reflectance moves towards shorter wavelengths. A change from normal incidence to 80° can cause a shift of λ_{max} towards shorter wavelengths by 7 nm or more, depending on the parameters chosen for the calculation. The spectrum reflectance changes occurring with altering angles of incidence are shown in Figure 9. And in the same figure are plotted observed values for wavelengths of maximal reflectance at difference angles to the normal to the tapetum lucidum of the rough dog.

We may expect the conditions of interference actually occurring in the tapetum lucidum to be fairly complex because the number of platelets differs from place to place, the spacing between adjacent platelets is subject to variation, and there are slight differences in the alignment of platelets. The variation in spacing alone is sufficient to produce the variety of colors to be seen in platelets in fresh tissue; and slight differences in alignment can introduce great complexities into the interference patterns because of the multiple reflections which can run along the length of the platelets and between adjacent platelets. It is to be expected that in the tapetum lucidum, organized as it is, the unsaturated overall coloration is produced by constructive interference throughout the visible spectrum. The resultant spectrum curve of reflectance is a composite, representing the combined reflectances at many wavelengths; it is continuous, and this fact suggests that there is a continuous play of constructive interference effects at all wavelengths. Higher reflection in the blue-green can be ascribed to a higher frequency of interlamellar spacing at favorable intervals, producing constructive interference in that region of the spectrum.

The repeated use of thin laminae formed of reflecting crystals to achieve interference effects and high reflectivity among different fishes is a remarkable instance of the same mechanism being pressed into service for diverse roles. Among teleosts, oriented reflecting layers in the skin contain crystals of two shapes, and these silvery and iridescent surfaces are concerned with concealing the fish (Denton and Nicol 1965a; b; 1966). In the same way, particular organs are concealed by silvery layers; *e.g.*, red muscle masses and the eyeball. The iris of the elasmobranch eye is loaded with crystals resembling those of the tapetum lucidum, and it has a shiny exterior. These surfaces are concerned with reflecting incident light in preferred directions so as to achieve certain effects in the visual field of other animals. Of a like nature are the iridescent spots borne by certain teleosts, the highly colored blue or green spots of the nuptial dress. Here again, the iridescence is produced by stacks of intracellular platelets or crystals (Kawaguti and Kamishima 1964; 1966). Certain colorations, especially green, are due to the combined effects of blue iridescence from iridosomes and the yellow of xanthophores (Fox 1953). The chorioidal tapetum lucidum, with its stacks of reflecting crystals, is similarly organized, but serves to reflect light into the retina of its possessor. Again, there is the reflector of the teleost photophore, which also has oriented reflecting cells containing stacks of small crystals (Bassot 1960; 1966). These are spaced at about the same intervals (150 to 350 nm) as those

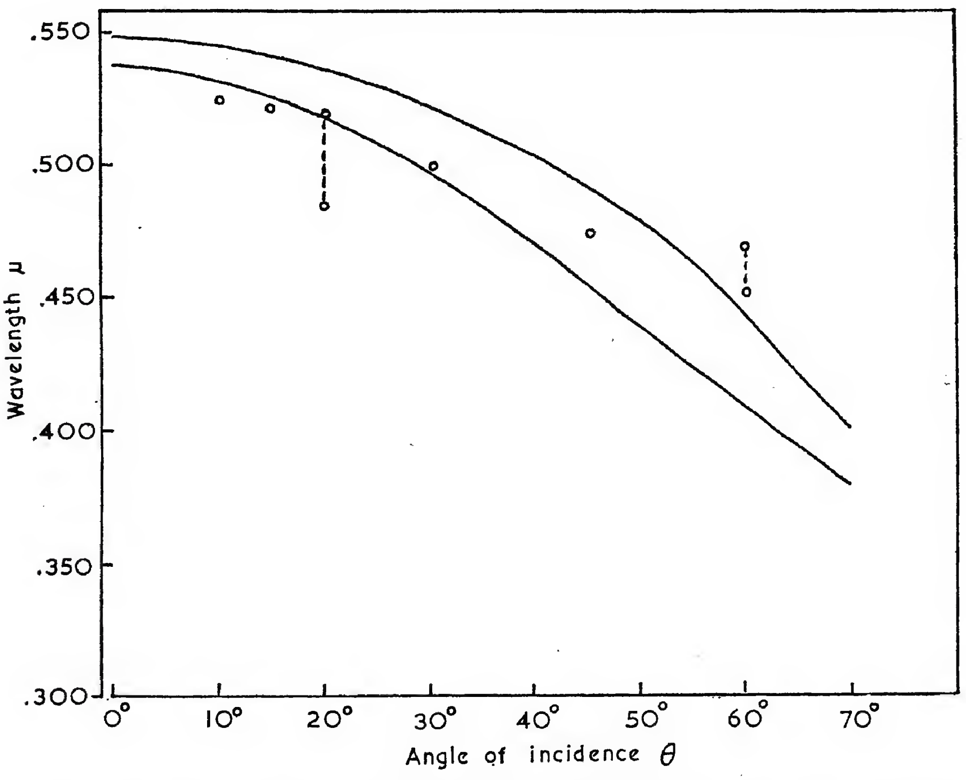

FIG. 9. Relationship of the angle of incidence to the color of a lustrous surface. The circles are measured values for λ_{max} of the reflectance of fresh tapeta of the rough dog (data from Denton and Nicol 1964). The upper line is Michelson's calculated curve for changing reflectance of equidistant laminae as a function of angle of incidence (n = 1.5). The lower line is a similar curve calculated for n = 1.33 (Michelson 1927).

occurring in the reflecting cells of the tapetum lucidum; they are concerned with reflecting light generated by the animal. Not only fishes have iridescent shiny layers, but the same kinds of surfaces are to be found in cephalopods (squid) about the eyeball and the ink sac, both dark organs that are thereby concealed. But in these animals the laminated layers producing the interference are of another kind, and they contain fine flexible needles, which are organized in regular patterns in the tissues overlying the organs.

ACKNOWLEDGMENTS

We wish to thank Mr. R. G. Maddock, senior scientific assistant at the Plymouth Laboratory for valuable technical assistance, and Mrs. Pauline G. West, Draftsman II of the Department of Zoology, The University of Texas, for doing some of the figures. Also, we thank Dr. David Paul Bloch of the Department of Botany, The University of Texas, for the loan of an interference microscope. Some of the expenses of this research were met by National Science Foundation Grant NSF-GU 1963.

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ADDENDUM

A closely analagous system to that of the tapetum lucidum is encountered in the eye of *Pecten* (described by Barber and Land 1966). The reflector of the eye of *Pecten* is multilamellar, made up of layers of thin crystals. These, 80.5 nm in thickness, have a refractive index of 1.80, much higher than that of the intervening cytoplasm. The latter space is 0.1μ. The optical path length of a crystal is 133, close to $\frac{1}{4}\lambda$, when $\lambda = 530$ nm, the wavelength of maximal reflectivity.

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Symposium

EFFECTS OF SUPERSALINE CONDITIONS ON AQUATIC ECOSYSTEMS

Sponsored by the Aquatic Ecology Section of the Ecological Society of America. Arranged by F. J. Schwartz and G. K. Reid. Seventeenth annual AIBS meetings of Biological Societies, 16 August 1966, The University of Maryland, College Park, Maryland.

PROGRAM

Copeland, B. J., The University of Texas, Port Aransas. Chemical and hydrobiological characteristics of hypersaline bays.

Carpelan, L. H., The University of California, Riverside. Invertebrates in relation to supersaline habitats.

Gunter, G., Gulf Coast Research Laboratory, Ocean Springs, Miss. Vertebrates and the supersaline habitat.

Bernstein, L., U. S. Salinity Laboratory, Riverside, Calif. Plants and the supersaline habitat.

Hoese, H. D., University of Georgia Marine Institute, Sapelo Island. Effects of higher than normal salinities on salt marshes.

*von Lossberg, L. G., Sheppard T. Powell and Associates, Baltimore, Md. Characteristics of desalting plant brines.

Whitney, R., Bureau of Commercial Fisheries, U. S. Fish and Wildlife Service, La Jolla, Calif. Increasing the utility of supersaline waters by the introduction of species.

*Pritchard, D. W., H. H. Carter and A. Okubo., The Johns Hopkins University, Baltimore, Md. Physical processes of movement and diffusion of supersaline effluent discharged into the coastal marine environment.

EDITOR'S NOTE

We are pleased to publish the proceedings of the symposium, "Effects of Supersaline Conditions on Aquatic Ecosystems." The symposium, sponsored by the Aquatic Ecology Section of the Ecological Society of America, was convened at the 17th Annual AIBS meetings of biological societies, College Park, Maryland, August 16, 1966. The symposium was arranged by Drs. F. J. Schwartz and G. K. Reid. The participants attempted to delineate environmental characteristics that may result from supersaline conditions.

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* Did not submit a manuscript.

The symposium was both pertinent and timely, considering the fact that man must obtain freshwater from the sea to supplement the increasingly contaminated freshwater supply. The brine waste from desalinization processes will constitute disposal problems of unknown magnitude and complexity. It behooves man, therefore, to make concentrated attempts to understand the problems manifested by supersaline environments.

We hope that the necessary adjustments made through our editorial process did not in any way affect the structure of the original symposium. Necessarily, editorial adjustments were made so that the manuscript would fit the style and objectives of these contributions. Minor editorial assistance was provided by Dr. F. J. Schwartz, for which we are appreciative. We assume responsibility for any editorial changes or comments.

The following are comments made by the conveners at the symposium. The papers appear in the order in which they were presented; however, some authors have changed the original title to fit later adjustments.

B. J. COPELAND, *Editor*

COMMENTS OF THE CONVENERS

GEORGE K. REID

Florida Presbyterian College
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More and more the world is becoming aware of the increasing unavailability of fresh water. The total water in the ecosphere is not diminishing, but rather the supply is being made unfit for re-use through pollution and other abuses of water on and in the lithosphere.

Some 60% of the earth's land area is classified as dry land, extremely arid, arid or semi-arid. Arid and semi-arid lands presently support only about five % of the world population. This situation is an extremely important limiting factor in human ecology.

Despoilage of fresh water is all-too-often relatively widespread and intensive in regions where the supply appears to be inexhaustible. There is also an increasing need for water in regions which are potentially capable of supporting overflow of human population increase. New sources of fresh water are being sought in order to cope with these problems.

Since man cannot increase the rate of the world hydrologic cycle or any phase of it on a massive scale, he must turn to the sea.

Today, over a dozen processes for desalting sea water have been found economically feasible and many of them are in actual operation. These processes include simple, single-effect distillation, freezing, electrodialysis, ion exchange, ultrafiltration, and osmionis (the osmosis of ions). (A 3.37 million dollar multistage flash evaporator producing 1.6 million gallons of fresh water per day was scheduled to begin operation in Key West, Florida in March 1967. This, obviously will be followed by many more and it is not the first by any means. It is the largest to date.)

Throughout history, it has seemed that major advances in human progress have been accompanied by some possibly adverse side-effects. (Examples are: the internal combustion engine and smog; mechanized agriculture and erosion.) Widespread utilization of desalination processes is inevitable; and it is certain that brine will be a by-product of the plants. A one million-gallon per day desalination plant will produce from 300,000 to 600,000 pounds of salt per day (United Nations 1965) and much of this salt will have to be disposed of as waste.

What types of brine disposal methods are available and what implications are there for ecological management?

"For plants on the sea coast, brine and cooling water disposal generally do not offer serious problems. Discharge into the sea does not require an elaborate head structure. The major consideration is that the discharge point be sufficiently far from the raw-water intake so that recirculation does not occur. This may require an extensive study of the direction and strength of currents. A sea coast brine disposal facility capable of discharging one million gallons per day will cost in the United States about $50,000, installed. The power requirements are about 5 hp per million gallons."

"Brine disposal for inland plants will be made up essentially of the brine from the process facilities and the blow-down from the cooling towers. The general three types of brine disposal could be used for inland plants: (i) discharge into a stream; (ii) discharge into ponds, and (iii) impounding of brine underground. Rarely are the first two possibilities practical. Discharge into a stream, even into a brackish stream, may cause chemical pollution problems which may extend for many miles downstream. Disposal into a pond ordinarily requires an excessively large area for surface evaporation. This method also demands a continual pond digging programme and could be applicable only to a very specific situation. Impounding of the brine through inverted wells is ordinarily required for inland plants."

"A hydrogeological survey of the site should be conducted for inland plants. A plant rejecting brine underground through wells may disrupt the water table and adversely affect freshwater supplies. These effects may extend over many miles. Discharge into streams and lakes can also result in similar problems which may extend over long distances. Injection of the brine into underground strata appears to be the most rational solution to inland disposal problems. If the brine well field is one mile from the plant and injection is at a depth of 1,500 feet, the completely installed facilities to handle one million gallons per day of brine would cost approximately $150,000 under United States conditions. Power requirements depend on the strata and are in the order of 150 hp for each million gallons of brine disposal" (United Nations 1965.) Each of the aforementioned possible methods of brine disposal will in some way and to some extent have effects on the ecology of streams, soil, bays, estuaries and coastline in the plant area.

This symposium was designed and organized to consider (1) some aspects of increased salinity in certain kinds of biotopes in the light of our present knowledge, (2) the nature of desalination operations, (3) the possible utilization of supersaline waters by introducing tolerant species and (4) the mechanisms of disposal of water in coastal waters. In the context of the symposium supersaline (hypersaline, hyperhaline) waters are those with salinity greater than 40 ppt.

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The topic of "The effects of supersaline conditions on the aquatic ecosystem" was conceived in the light of several factors. These factors were: 1) increased demands for freshwater by the burgeoning world community (causing everyone to become conscious of this need for basic sustenance;) 2) desalinization plants have, are, or will be established throughout this and other countries in the near future, without any prior knowledge of the implications of such ventures; 3) knowledge is lacking concerning the effects of such operations on the biota of coastal areas; 4) knowledge is lacking on how these operations could possibly be put to constructive use; and 5) it seemed best to stop, look, and listen before too many events took place which we may eventually regret.

The descriptive term "supersaline" was dreamt up. Reference to Barnes', Vol. 2, Biology and Annual Review (1964) or the Symposium on Classification of Brackish Waters (1958) yielded almost no agreement between the world's scientists when designating various salinity regimes. Waters up to 40 ppt seemed to have fairly uniform agreement and usage; those above did not. The supersaline condition has no real meaning other than it describes a saline environment with a concentration of ions greater than 40 ppt salt and one which may be natural or man-made.

The natural high saline habitats are such places as the Salton Sea, the Laguna Madre, the Caspian Sea, and some sections of the Black Sea. The man-made ones are those that will occur near desalinization operations, or megalopolis constructions. In each case, faunas exist today. The questions that now arise are: What will happen when high saline brines or sludges from desalination or other operations are introduced; will species, old or new, tolerate these conditions; will others move in if the present fauna is altered; will new species be created; or will the area be made sterile, etc.?

Another aspect of the problem is man's activities other than saline plant operations. Will his activities of land fill, contamination, and damming of lagoons, marshes, coastal estuaries, etc. in this and other parts of the world create supersaline environments? Areas, removed by man from the natural exchange between the land and the sea, are creating supersaline habitats and causing changes for good, better, or worse.

A third reason for creating the Symposium is that although there have been two such symposia in the world, one in England and one by the Office of Saline Waters in this country, few efforts have attacked the problem as in our Symposium at College Park, Maryland. One or the other existent symposia simply approached this topic from an engineering aspect or what would be the effects of various molecules when discharged into a habitat or how one should go about setting up the commercial use of saline basins, and/or using its waters and its by-products. It is hoped that by stopping to look, see, and listen, we will be able to approach the theme of the Symposium in a better light and that we will be able to make more progress than in the past.

Typical of all symposia, too late one can always think of other aspects that should have been discussed. We, here, are approaching the habitat, ecology and species interaction aspects of this problem. It would have been well to have included also the physiological and molecular aspects of a species as it reacted to the supersaline habitat. Whether cells or organ systems will tolerate the new supersaline environment is linked to these levels of biology where great studies have recently taken place. Examinations must now await another day.

We must not, however, wait too long for that other day. Man is a very capable species. He can create, he can preserve, and he can and does destroy. Let us hope that he has the wisdom to stop, look, and listen before he ventures into or creates this new and hostile environment—the supersaline habitat. Little of his natural waters, fresh and marine, remain. These are his last frontiers; he must use them wisely.

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ENVIRONMENTAL CHARACTERISTICS OF HYPERSALINE LAGOONS

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ABSTRACT

Some chemical characteristics of naturally occurring hypersaline lagoons were discussed. The possible impact of desalinization plant effluent on the existing estuarine environments was integrated into the characteristics of natural hypersaline environments. Data concerning the salinity and species diversity of a hypersaline Mexican lagoon are given for a five-year period. Differential ion precipitation, alkalinity, pH and oxygen saturation versus salinity are discussed for evaporated seawater, Diurnal characteristics of dissolved oxygen, carbon dioxide and temperature in natural hypersaline waters are discussed.

INTRODUCTION

In the arid coastal regions of the world, where evaporation exceeds freshwater input, coastal waters become hypersaline; *i.e.*, salinity above 40 gm/kg (ppt). Hypersaline conditions occur naturally in the Laguna Madre of Texas, although a large volume of normal seawater has been made available with the completion of the intracoastal waterway, preventing the establishment of extreme hypersaline conditions that once were possible (Behrens 1966). The Laguna Tamaulipas in northeastern Mexico, another hypersaline lagoon, is the only remaining coastal bay adjacent to our country that resembles the primeval conditions which existed prior to the rise of extensive industrialization and civilization along the Texas coast.

In some coastal regions of the world, salt is harvested from solar evaporating ponds. Water is commonly siphoned from the adjacent sea and evaporated while being transferred from pond to pond (Baas Becking 1931). The transfer from one pond to another at a certain salinity level affords the removal of certain unwanted ions from the final salt harvest. Salinity attained in those ponds reaches the level of sodium chloride saturation, which is approximately 300 ppt.

Hypersaline environments pose several problems to aquatic organisms due to many factors. The most obvious of these factors is osmosis, although many other stresses exist simultaneously. In the following data, some special chemical characteristics of hypersaline waters will be shown and the implications of their effects on organisms will be discussed. Special problems include: salinity, differential ion precipitation, pH, dissolved oxygen and temperature.

SALINITY CHANGES IN NATURE

Some degree of hypersalinity is almost always present in bays and lagoons in arid coastal regions, except during wet years (Behrens 1966). With the installation of the intracoastal waterway (removal of permanent sills) in the Laguna Madre of Texas, salinities there have not exceeded about 75 ppt and probably never will. Before then, however, salinities were higher than 100 ppt on several occasions (Collier and Hedgpeth 1950). In the Laguna Tamaulipas, just down the coast in Mexico, salinities during the past few years have reached very high levels.

Salinity data, collected over a five-year period, from the Mexican Laguna are shown in Figure 1. Plotted against the salinity data are data expressing the

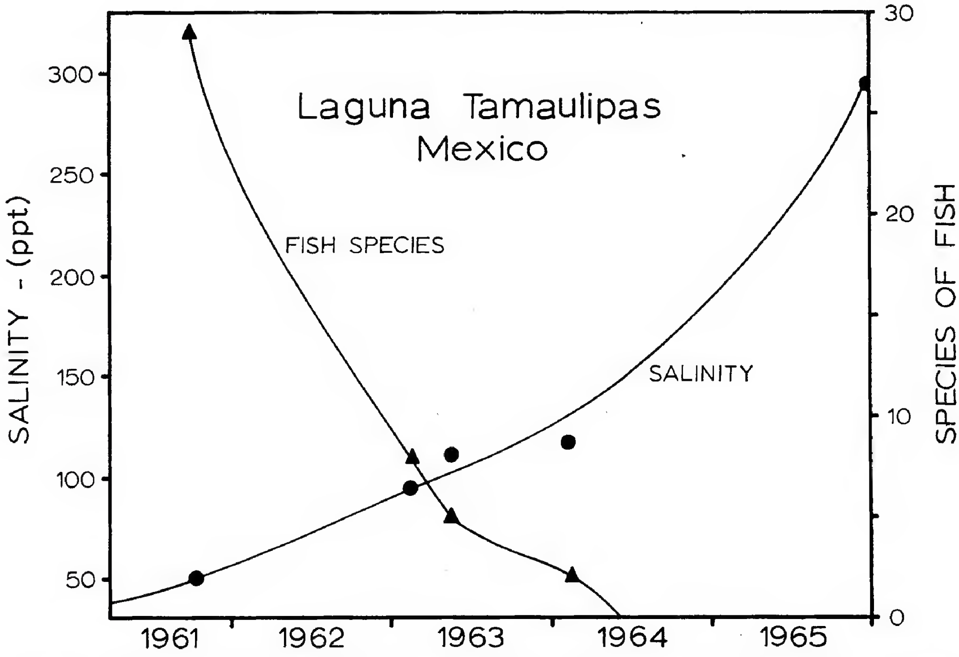

Fig. 1. Salinity in parts per thousand and number of fish species in the Laguna Tamaulipas, Mexico, during 1961 through 1965.

species diversity of fishes found in the lagoon. In October 1961, when the salinity was 51 ppt, there were 29 species of fishes, including the ones commonly found in the more saline bays of Texas; however, by 1963 the number of species had decreased to eight in February and to five in March and the salinity had increased to 95 and 110 ppt respectively. In February 1964, after the salinity had varied between 110 and 120 ppt for almost a year, only the fishes *Cyprinodon variegatus* and *Menidia beryllina* remained. Even these hardy beasts disappeared when the salinity reached 140 ppt. After a salinity of 150 ppt was reached *Artemia salinas* was found to be the only animal present. Even they had disappeared by December 1965 when the salinity was 295 ppt and sodium chloride was precipitating, although there were large wind-rows of brine shrimp eggs on the windward shore.

DIFFERENTIAL ION PRECIPITATION

In addition to osmoregulatory problems encountered in hypersaline waters, the aquatic organism must also contend with imbalance of cations and anions in the environment. The theory of constant proportions of salts in seawater is an old one and means that ionic concentration varies only with dilution but that the ratio of one ion to another remains the same. Organisms have become adapted to this constant ratio in seawater and minor variations in estuaries.

When seawater evaporates, however, a differential precipitation of salts occurs, depending on concentration of a particular salt and the solubility of the salt. This poses a problem to many organisms and it is further complicated by the fact that the solubility of any given salt is different in the presence of other salts. Chemical interaction between two or more ions may take place with the separation of double or triple salts.

Differential salt precipitation occurred during the evaporation of seawater, as shown in Figure 2. The horizontal lines indicate the salinity at which separation of a particular salt began. Ferric oxide and calcium carbonate separated at just below 70 ppt; borates at 120; calcium sulfate at 150; sodium chloride, magnesium sulfate, and magnesium chloride all separated at 290; and sodium bromide and potassium chloride were still in solution at 300 ppt. The implications here are that if an organism could solve the osmoregulatory problems it would be faced with an environment far different than the one to which it was adapted. I am specifically referring here to the algal species that make up the loosely organized mat that exists on the bottom of hypersaline lagoons. It would appear that to survive the algae must live on the bottom since many of the necessary salts are no longer in solution in water and must be taken from the bottom sediments where they have settled. Any floating organisms would have serious existence problems, although *Dunaliella salina* are able to survive in salinities less than 100 ppt.

Closely related to differential ion precipitation is the problem of alkalinity. As shown in Figure 3, the relationship between alkalinity and salinity is not linear. For seawater, the ratio of alkalinity to salinity is about 0.068 milli-equivalents per liter per ppt. For Gulf of Mexico water, this ratio seems to hold true between 35 and 120 ppt (Fig. 3). However, at 120 ppt the alkalinity-salinity ratio drops very suddenly to about 0.041 milli-equivalents per liter per ppt. Theoretically,

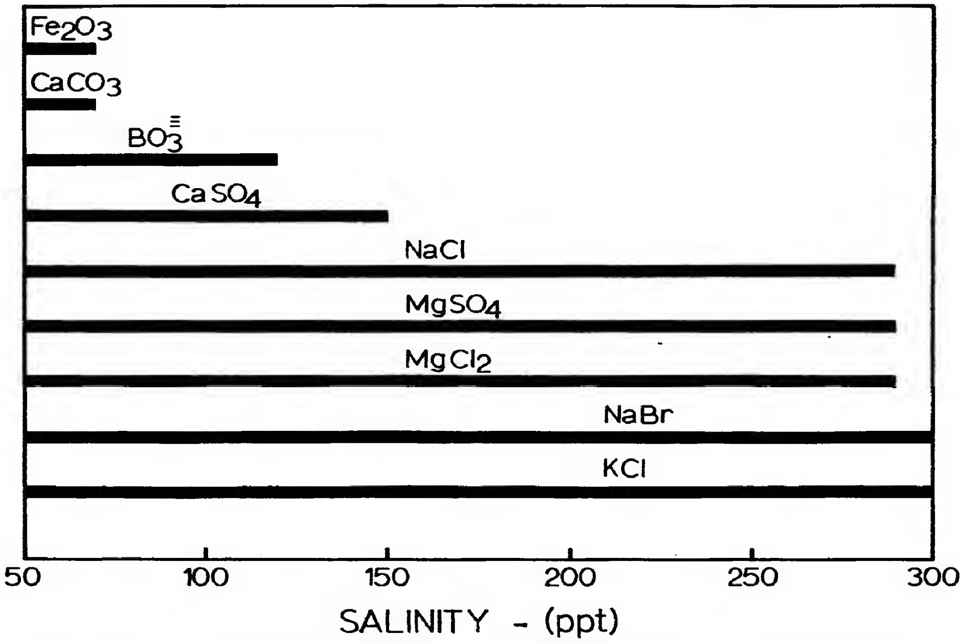

Fig. 2. Precipitation of salts during evaporation of seawater (modified from Thompson and Robinson 1932). The end of the bar indicates the salinity at which precipitation first occurred.

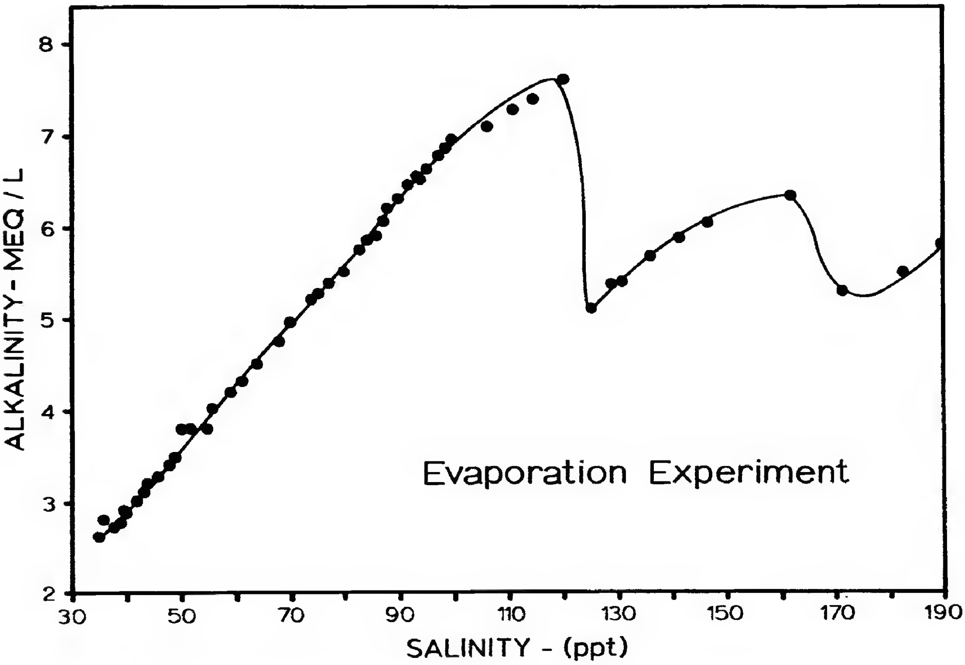

Fig. 3. Alkalinity in milli-equivalents per liter during the evaporation of seawater.

the alkalinity-salinity ratio should drop at about 70 ppt since that is where the carbonates begin to separate from the aqueous solution. It is not clear if there is enough carbonate left in solution or if the borates replace them. After the tremendous drop at 120 ppt, alkalinity again increases with salinity, but at a lesser ratio to salinity. The next drop coincides with the separation of calcium sulfate, with another increase as salinity increases. The sudden decreases in alkalinity may also be explained by the fact that perhaps not all of the carbonate separates; *i.e.*, there is a differential precipitation of carbonate with increases in salinity. Whatever the cause, an organism subjected to such changes encounters tremendous changes in the buffer system of the environment.

HYDROGEN ION CONCENTRATION

As the ratio of carbonate to bicarbonate increases, the pH, which depends upon these ratios, increases and vice versa. During the evaporation of seawater, carbonates increase until saturation is reached at about 70 ppt (see previous section); thus, the average pH should increase, reaching a peak at about 70 ppt.

As shown in Figure 4, pH increases significantly in the early stages of evaporation of seawater. The pH values for water of 50 through 90 ppt as shown in this figure were measured in the Alviso Salt Ponds, San Francisco, California by Carpelan (1957). Generally speaking, the pH in natural waters during evaporation increases to a value above nine just before saturation with calcium carbonate

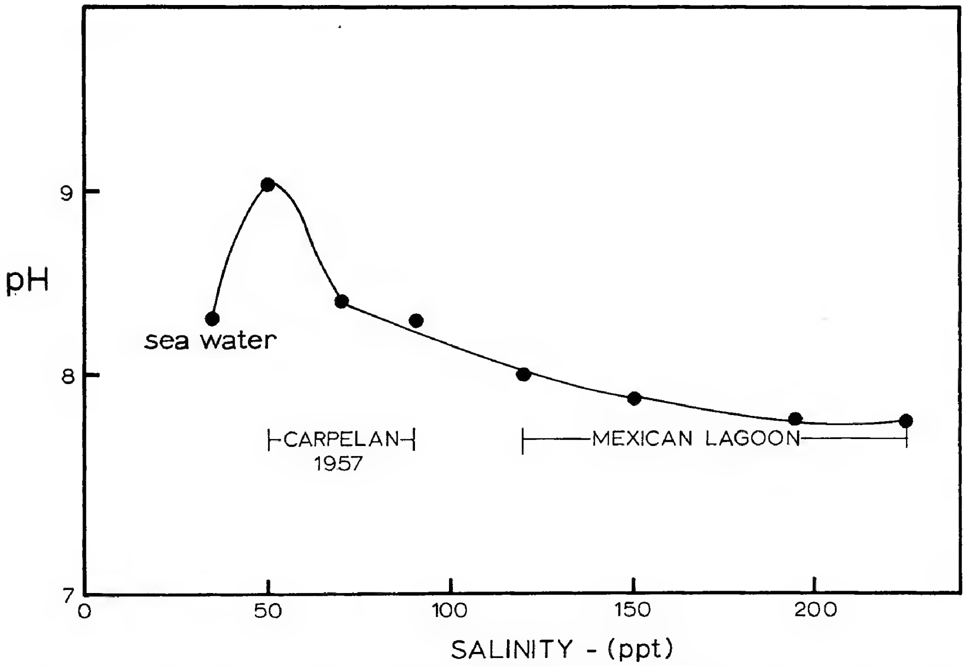

Fig. 4. Average pH of seawater at various salinities in natural bodies of water. The pH at 50 through 90 ppt salinity were taken from the data of Carpelan (1957), salt-evaporating ponds near San Francisco, Calif. The Mexican Lagoon refers to data from the Laguna Tamaulipas, Mexico, 1964–65 (From Copeland and Jones 1965). Seawater is Gulf of Mexico water.

and decreases rapidly when carbonate precipitation occurs. A general decrease follows further evaporation until equilibrium is reached at about pH 7.8, when the salinity reaches about 200 ppt. Presumably, this is in agreement with leveling of alkalinity above 200 ppt.

There is a diurnal change in pH, even in very saline waters, presumably because of photosynthesis and respiration of the limited assemblages of algae and bacteria. An example of diurnal pH variations and rate-of-change of carbon dioxide is shown in Figure 5 for water with 150 ppt salinity. The pH ranged between 7.78 and 7.95, with an increase during daylight and decrease during darkness. A discussion of photosynthesis and respiration in hypersaline waters has been presented in a previous paper (Copeland and Jones 1965).

The high pH at salinities between 50 and 70 ppt may have many adverse effects on organisms that are able to survive the osmotic problems. Above pH 9, hydrogen ion concentrations may have toxic effects on some organisms that are adapted to a lower and more constant pH of seawater, especially the larvae.

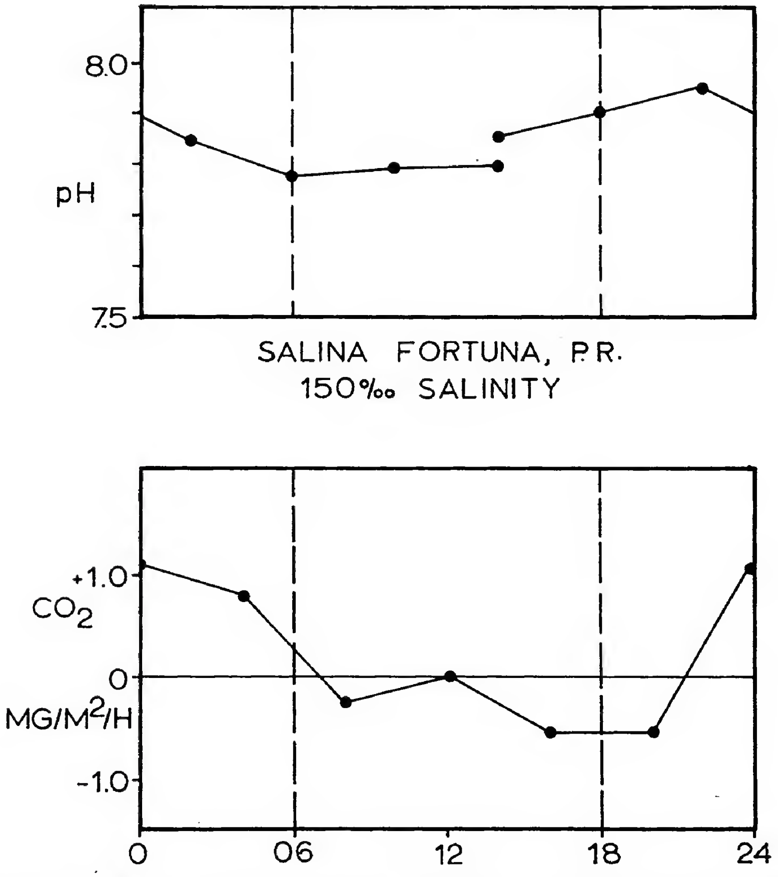

FIG. 5. Diurnal change of pH in a salt evaporating pond, Salina Fortuna Salt Works, La Parguera, Puerto Rico, 150 ppt salinity. The upper graph is the pH and the lower graph is diurnal carbon dioxide rate-of-change. (From Copeland and Jones 1965).

Photosynthesis by some algae may be limited because carbon dioxide concentration in seawater is almost zero at pH 9 (Moberg *et al.* 1934). Thus, algae not able to utilize bicarbonate will not be able to photosynthesize. With the effect of evaporation of seawater in changing the carbonate-bicarbonate ratio and influencing the pH, there is an abrupt change in flora that are able to photosynthesize in these waters. This is the basis that the primitive salt makers, since biblical times, have utilized to determine when evaporating water should be drained from one evaporating pond to the next in order to leave behind undesirable ions (Baas Becking 1931). Scofield (1961) presented colored photographs demonstrating the beautiful coloration of flora that characterizes waters of different salinity in the salt evaporating ponds of Haiti.

DISSOLVED OXYGEN

The fact that the solubility of oxygen in water decreases when the salinity of that water increases, if other factors are kept equal, has been well established. Formulae developed by Gameson and Robertson (1955), Truesdale *et al.* (1955) and Truesdale and Gameson (1957) have been used for calculating solubility of oxygen in seawater relative to variations in salinity and temperature, but such formulae are appropriate only within a small range; *i.e.*, zero to 40 ppt. As shown in Figure 6, extrapolation of the widely used formulae indicates that water above 170 ppt salinity would be devoid of oxygen unless supersaturation was possible. Obviously, this extrapolation is incorrect, because brine shrimp are able to live in waters of higher salinity.

Since data were not available for saturation of water above 40 ppt salinity with oxygen, experiments were designed to provide dissolved oxygen saturation values for hypersaline waters during a previous study (Copeland and Jones 1965). Water samples of 95 to 220 ppt salinity from the Laguna Tamaulipas, Mexico were slowly stirred while held at constant temperatures. Temperatures were 25 and 37 C, the range encountered during the study. After equilibration with the atmosphere (760 mm Hg) the oxygen concentration was measured by the Alsterberg modification of the Winkler technique (APHA 1960). Saturation was verified by comparing the output of a galvanic cell oxygen analyzer in the hypersaline water with the output in pure water (the galvanic cell oxygen analyzer relies upon the partial pressure of oxygen in water, Mancy and Westgarth 1962).

The data obtained via the above experiment were significantly different from the extrapolated values using the formulae of Truesdale *et al.* (1955). The experimentally obtained saturation values formed a curvilinear relationship (Fig. 6) with a tendency to level off near 200 ppt salinity.

The theory of the oxygen saturation relationship shown in Figure 6 (*i.e.*, a change in slope of the line in a positive direction) can be found in any good physical chemistry text (*e.g.*, Glasstone 1941). Since the escape-tendency of the gas is increased (and hence its solubility decreases) because of the competition of the electrolytes for water molecules, the differential ion precipitation (*see* Fig. 2) that occurs above 70 ppt salinity would alter the linear relationship extrapolated from calculations in normal seawater. Theoretically, the curve for oxygen saturation values should undulate, with the maxima occurring just after the precipitation of a salt and the minima occurring just before the precipitation of a salt. Unfortunately, data for oxygen saturation values were not taken at close enough intervals in salinity to test that theory. Furthermore, the extrapolated straight line should be similar to the real value until 70 ppt salinity when the first salts begin to precipitate (Fig. 2).

Another factor is that as concentrations of salts occur their attraction to one another lessens the competition for the water molecule, thus permitting a greater solubility of gases. This would tend to smooth the curve for oxygen saturation values and add more validity to the curvilinear relationship shown for hypersaline waters in Figure 6. Further experimentation is needed, however, before this can be viewed as being more than fortuitous.

There is a diurnal fluctuation in dissolved oxygen concentration in hypersaline waters encountered in nature, at least to 225 ppt salinities (Fig. 7). This diurnal fluctuation results from photosynthesis and respiration by algae and bacteria as well as the oxidation of organic material deposited by evaporation. The daily amplitude in the fluctuation of oxygen concentrations is small, presumably the result of reduced solubility of oxygen at the salinities encountered. Copeland and

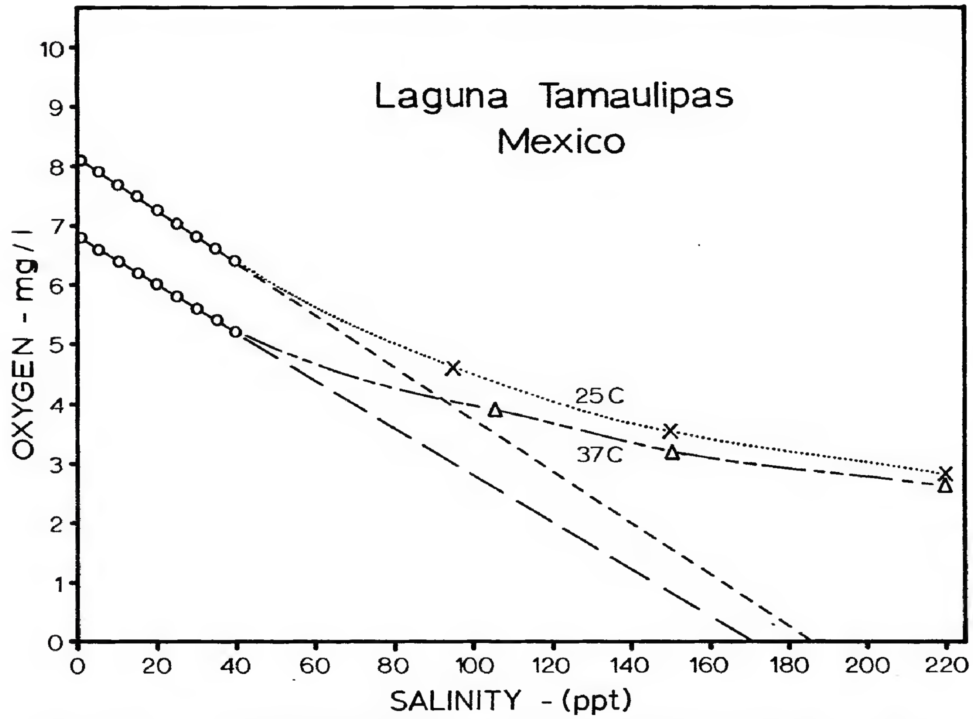

Fig. 6. Saturation values of dissolved oxygen versus salinity. The open circles indicate 100 % saturation in mg/l, using the solubility constants of Truesdale *et al.* (1955), at 25 and 37 C. The straight lines indicate theoretical saturation when the data at lower salinities are extrapolated. The open triangles and crosses indicate 100 % saturation as measured in water from the Laguna Tamaulipas, Mexico, at various salinities.

Jones (1965) have discussed photosynthetic productivity in hypersaline waters in some detail.

In the Laguna Tamaulipas, Mexico, and the salt evaporating ponds in Puerto Rico, oxygen concentration never reached zero (Copeland and Jones 1965). However, in an evaporated tidal pool on St. Joseph Island, Texas, the oxygen concentration was zero during nighttime and almost 300% during midday (Fig. 8). This pool was unique as its inhabitants were both pink bacteria and a species of *Chlamydomonas*. It is possible that the *Chlamydomonas*, which has not been found in waters of higher salinity, was able to carry on considerable photosynthesis during daylight and to lie dormant during nighttime.

The lower curves in Figures 7 and 8 indicate the diurnal rate-of-change in oxygen concentration. The amplitude of the rate-of-change curves were of similar magnitude as curves for waters of lesser salinity (Copeland and Jones 1965).

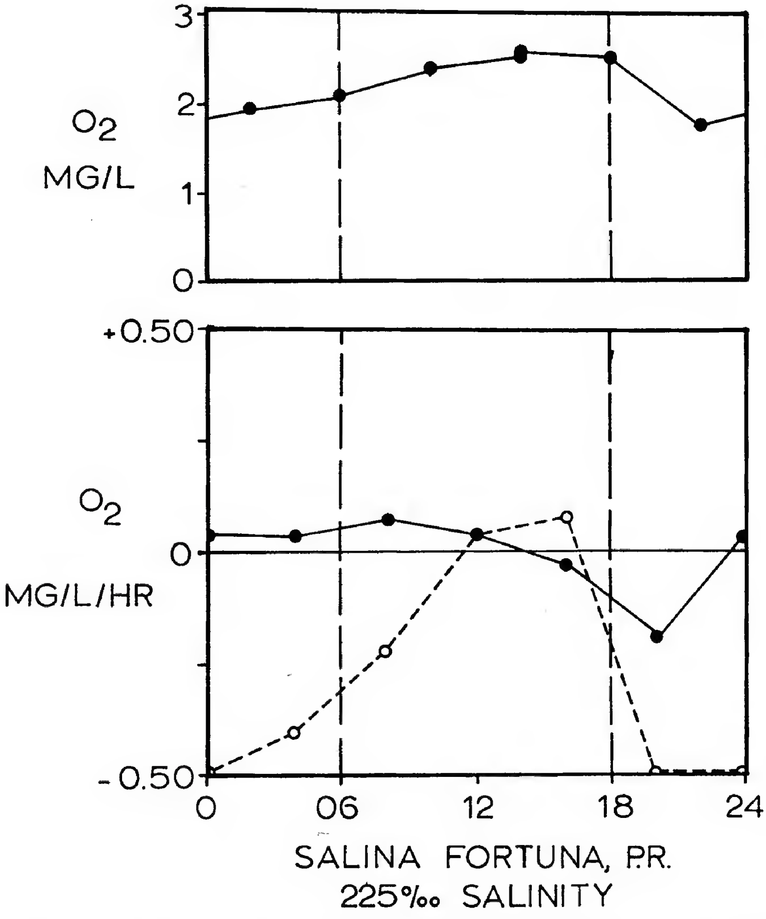

Fig. 7. Diurnal change of oxygen in a salt evaporating pond, Salina Fortuna Salt Works, La Parquera, Puerto Rico, 225 ppt salinity. The upper graph is the dissolved oxygen in mg/1 and lower graph is diurnal oxygen rate-of-change. The dashed line in the lower graph indicates the diurnal oxygen rate-of-change as corrected for diffusion. (From Copeland and Jones 1965).

TEMPERATURE

Pure water has a specific heat of one. As the concentration of dissolved salts increase, the specific heat decreases. For sea water at 17.5 C and 35 ppt salinity the specific heat is 0.932. This suggests that less heat is required to warm a given volume of salt water than to warm the same amount of fresh water. It follows, therefore, that waters approaching the salinities encountered in hypersaline lagoons around the world would exhibit tremendous fluctuations in diurnal temperature. As shown in Figure 9 there is a large fluctuation in temperature in Laguna Ta-

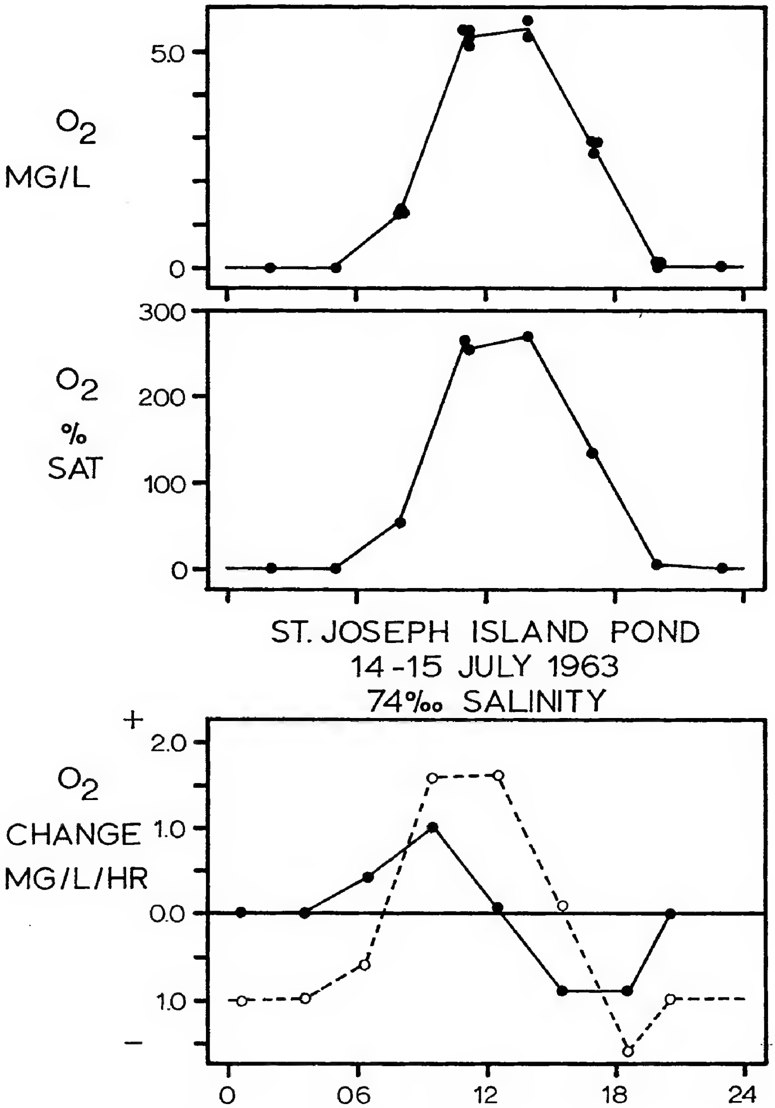

FIG. 8. Diurnal change of oxygen in a tidal pool on St. Joseph Island, Texas, 74 ppt salinity. See Figure 7 for explanation. (From Copeland and Jones 1965).

maulipas, Mexico, even during early spring. During late spring, the water temperature often exceeded 37 C during mid-day.

Since many chemical reactions are temperature dependent, the large fluctuation and high mid-day temperatures in natural hypersaline waters are of vast importance to the enviromental characteristics of such waters. The solubility of oxygen is less at high temperatures, regardless of the salt content; therefore, water will hold less oxygen during the day than during the night. Additionally, the precipitation of various salts as discussed previously is affected by variations in temperature.

GENERAL CONSIDERATIONS

With the decrease in water flowing from rivers of our country into the estuaries there is a general increase in salinity in coastal waters, especially in the arid regions of south Texas. These estuaries are shallow and evaporation rates are high, so that in the absence of dilution by fresh water the estuarine waters will continue to become more saline.

With the coming prevalence of desalinization plants in coastal areas, coastal waters in the proximity of such plants are quite likely to become hypersaline. The brine left after the extraction of water from seawater will undoubtedy be diverted into coastal waters. Since this brine will exhibit some of the characteristics discussed in the previous sections, coastal waters will tend to exhibit similar characteristics. With the combination of reduced fresh water for dilution and the increase in man-made brine, the hypersalinity problem in coastal waters will be compounded.

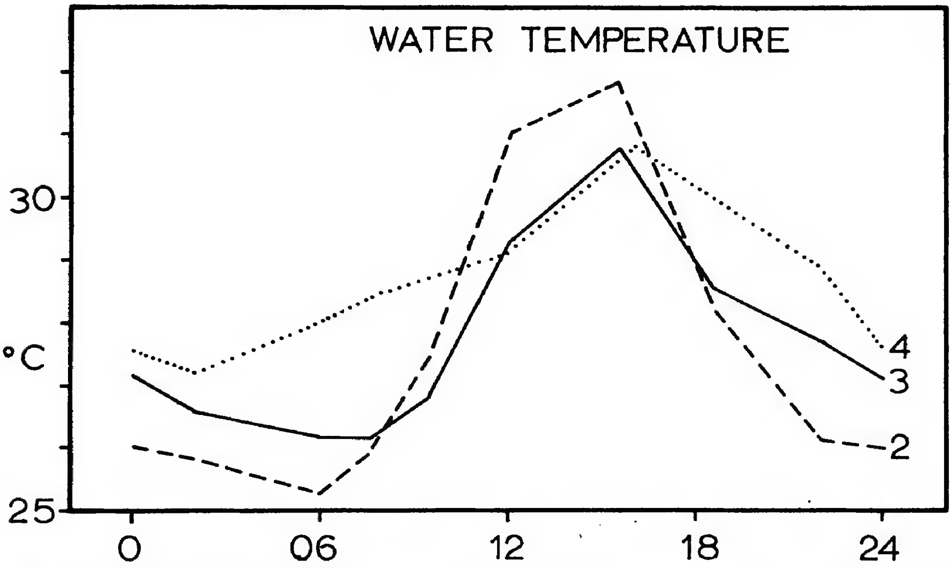

Fig. 9. Diurnal temperature range for three stations in Laguna Tamaulipas, Mexico, 115 ppt salinity.

As has been pointed out, the environmental characteristics of the few hypersaline waters existing in nature are harsh indeed. It is probable that the situations discussed above will provide much the same environment. Even if organisms are able to adjust to the osmotic problem, they are faced with an imbalance of ions, lower oxygen concentrations, adverse pH, strange buffer systems and large temperature fluctuations. It is not surprising, then, that very few organisms are or will be found in such difficult environments.

ACKNOWLEDGMENTS

This study was supported by a grant of funds from the U. S. Public Health Service (now Federal Water Pollution Control Administration), WP 00204. The data for Figure 3 were taken with the assistance of Dr. Robert J. Beyers and Mrs. Rita O'Donnell. Mr. William Gillespie provided technical assistance and constructed several of the Figures. Dr. Patrick L. Parker provided theoretical discussion and many helpful hints regarding the interpretation of chemical and physical data.

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INVERTEBRATES IN RELATION TO HYPERSALINE HABITATS

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ABSTRACT

Organisms in supersaline waters have been of interest for their distribution and also osmotic and ionic regulation. Few physiological generalizations are possible. The organisms in waters other than fresh or marine have been of interest as "indicators" to characterize waters. Lack of stability characterizes brackish and supersaline waters. The environment is constantly changing and the organisms must be sufficiently euryhaline to include either fresh or marine waters within their range of tolerance. Restriction to some range of salinity other than fresh or marine would be an adaptation to extinction. The species tolerating supersaline conditions attain tremendous numbers. Few productivity studies have been made, apparently in the belief that generalizations cannot be made in studying a transitional community; that is, only a stable community can give general answers to questions regarding productivity and utilization of energy within an ecosystem.

INTRODUCTION

The chapter on Estuaries and Lagoons in the treatise on Marine Ecology and Paleoecology by Hedgpeth (1957) probably has had the greatest influence on what I have to say. I was also influenced by recent reading of the papers presented at the 1959 Moscow Symposium on the Origin of Life on Earth. A review by Kinne (1964) provides a summary of the physiological aspects of our subject. Kinne also defines the environment of concern. He limits hypersaline to the range from 40 to 80 ppt; he considers higher concentrations to be brine. Brines have a peculiar fauna; hypersaline waters do not. That is my first point: we are not concerned with an extraordinary fauna. According to Hedgpeth (1957) "there is no characteristic endemic hypersaline component." This is similar to the view of Day (1951) who, in commenting on estuaries, said "no peculiar hypersaline fauna has been found and those organisms living at salinities of 40 to 60 ppt are merely forms of unusual tolerance." We might quibble whether their "unusual tolerance" does not make them peculiar, but Days's point is well taken. It is wide tolerance that sets the organisms of lagoons and estuaries apart from freshwater and marine organisms. And it is tolerance to a generally unstable environment, one subject not alone to fluctuations in salinity, but to wide changes in other environmental factors as well.

Kinne and Day agree on 40 ppt as the upper limit of marine and the threshold salinity for hypersaline habits. However, one places the upper limit at 60 ppt, the other at 80 ppt. I have tended to use 70 ppt, which is 2X seawater concentration. This range from 60 to 80 ppt is significant, though the exact limit is not, because it varies with temperature and with pH. It corresponds roughly to the concentration at which there is upset in ionic ratio of sea water because of precipitation of the least soluble compounds.

And here we find the reason for differentiating inland mineral waters from coastal brackish waters. The differentiation at first glance seems illogical because there are inland mineral waters (so-called chloride waters) which resemble the ocean in ionic proportions, and which support a marine biota when in the marine range of salinity. The Salton Sea of southeastern California is in this category. When such waters or marine waters evaporate to greater-than-seawater salinity, the same euryhaline marine species can exist in both the inland chloride water, and in hypersaline seawater; but only for limited increases in salinity, to about 70 ppt, before precipitation of salts begins. If evaporation continues and the salinity approaches that of the Great Salt Lake, for example, there is loss of the marine component. In the resulting brine will be found the same bacteria, the same algal flagellates and other protists, and same insects and the brine shrimp, whether the water originated in a coastal lagoon or in an inland lake.

The difference between the marine-derived euryhaline biota and the brine organisms is not due entirely to distributional problems nor to origin of the water near to or distant from the sea, but results from differences either in ionic proportions or increases in osmotic pressure, or both. After the least soluble compounds, $CaCO_3$ and $CaSO_4$, have precipitated, there remains the brine which consists of the more soluble sodium, potassium and magnesium halides. As seawater becomes concentrated there are two chemically-defined boundaries in the supersaline region, plus one at saturation with NaCl. One boundary is at about 2X seawater salinity at which concentration $CaCO_3$ begins to precipitate visibly at low pH, and the other is at about 5X seawater salinity at which all the $CaCO_3$ has come out of solution and CaSO4 begins to precipitate. The next big change comes at about 8X seawater when NaCl, $MgSO_4$ and $MgCl_2$ also come out of solution and only NaBr and KCl are left.

Beginning at 2X seawater there is an upset in ionic proportions; after a concentration of about 5X is reached, the Ca is gone and what remains is a rather unbalanced physiological medium and one of high osmotic stress. Thus we might expect upper limit of about 70 ppt for euryhaline marine organisms. This is the limit we are concerned with today. It is the limit used by crab physiologists whose familiar curves relating blood and media freezing point depressions have the maxima of their salinity scales at 2X seawater concentration or less, *e.g.*, Figure 1 from Kinne (1963). The brine organisms are tolerant to a different order of magnitude; they thrive in water up to saturation with NaCl.

In addition to inland chloride waters (which resemble ocean waters) there are inland mineral waters low in chlorides but high in carbonates or sulfates. And there are combinations of these in which ionic ratios different from the sea occur at low salinity. These and brines are being ruled out of consideration today. Among the questions of concern are two physiological ones: those of osmotic stress and of ionic ratios. And there is the basic evolutionary question of the source of origin of the hypersaline fauna. Why can so few organisms live in brines, and why do so few live in the range from 70 to 150 ppt, a range with relatively little upset in ionic ratio?

The fauna of the hypersaline range, 40 to 80 ppt, can be characterized as consisting of estuarine organisms, which are also known as brackish water species and/or as euryhaline marine species It seems that organisms capable of tolerating increases in salinity are also capable of tolerating reductions in salinity relative to marine water. This was observed and commented upon by Ferronnière (1901) more than 60 years ago.

Another pertinent observation made long ago is that in estuaries and hypersaline lagoons the number of species is reduced relative to the number of marine species; but that species thrive and attain large numbers of individuals. This statement is no different from what could be said of the organisms of any unstable environment. Only generalized "pioneer" or "colonizing" species are to be expected where conditions fluctuate and are too unstable to allow succession to occur. This type of community is called "pioneer climax" by plant ecologists. The community begins and ends at the pioneer stage in a situation analogous to arctic tundra or to certain desert habitats.

A principal effect of the hypersaline environment on the ecosystem is to simplify the food relations. Food chains may be diagrammatic in simplicity with but one species per trophic level. The relatively few species that thrive under these extreme conditions build up large numbers, presumably for lack of competition.

The reduced number of species is emphasized because this paucity leads to the impression that these are specialized forms. They can indeed be viewed as specialized—their specialization being wide tolerance to salinity and to many other environmental factors. However, this is a matter of opinion or of point of view, because their wide tolerance makes these organisms generalized. They are euryhaline, eurythermal, etc., in contrast to specialized stenohaline species which are limited to narrow ranges. The generalized species capable of exploiting the hypersaline waters have less competition in the fluctuating environment because most modern species are specialized for life in one or the other of the two stable aquatic environments, freshwater or seawater.

The instability of hypersaline and brackish waters concerns more than salinity.

The existence of hypersaline waters, other than those of mineral springs, requires that evaporation exceed precipitation, at least seasonally. So hypersaline waters occur at latitudes favorable for evaporation, or, in the case of inland waters, in desert-like locales such as rain-shadows of mountain ranges. Evaporation is favored by full insolation; high daytime temperatures and diurnal fluctuations greater than in the oceans are to be expected. There is also a necessity for shallowness; salinity cannot increase markedly without a favorable surface to volume ratio. (Shallowness may also have an evolutionary implication, discussed later.) In our present context, shallowness leads to fluctuations in several factors in addition to temperature, including pH and concentration of dissolved oxygen. These biogenic changes are pronounced because primary productivity is high per unit volume. Nutrients are brought into the shallow basin and rapid recycling of nutrients is favored by proximity of bottom to surface.

FAUNA

Data from this environment are understandably scarce. There is a reluctance to invest time in a long-term study of a body of water where the hypersaline condition could end momentarily with an unusually high tide or with a flood of freshwater. There are few data on such matters of concern to the ecosystem as energy relations or population dynamics. An additional drawback to the study of hypersaline environments is inability to generalize from any particular body of water. For this reason we have to recognize that in this area there are differences of opinion. For example, there are two kinds of hypersaline lagoons. I have studied small ones which are the mouths of intermittent streams which become completely cut off from the sea by sand bars. And these may evaporate to dryness. Such lagoons are quite different from those such as the Leguna Madre of the Gulf coast, which are arms of the sea, permanently open to the ocean. The salinity of the Laguna Madre seldom exceeds 70 ppt. The organisms in it are euryhaline marine organisms. Some of them enter and leave and are not really established residents. An arm of the sea is a more open system than the ecosystem encompassed by an enclosed lagoon. Another difference is that there is relative stability in the larger arms-of-the-sea type of lagoon.

The Sivash, somewhat comparable to the Laguna Madre, is connected to the Sea of Azov. As described by Zenkevitch (1957) the Sivash has a rather stable salinity gradient from 11 to 150 ppt. Four marine (Black Sea) invertebrates occur at salinities above 70 ppt, whereas invaders from the opposite direction, from land and from freshwater, consist of what the Russians call the ultrahaline fauna of insects and branchiopods. *Artemia* penetrates from the more concentrated end of the Sivash toward the dilute end to about 75 ppt;.*Ephydra* larvae to 50 ppt; Chironomid larvae occur to a low salinity of 20 ppt.

The fauna of inland brines includes insects, which are of terrestrial origin and possessing aerial means of dispersal, and branchiopods, which are of fresh water derivation and which have resistant stages. The euryhaline marine species have neither winged means of dispersal nor resting stages. The lack of special means of dispersal or of means of getting past periods of desiccation emphasizes the need for a range of tolerance that includes, as a home base, one or the other of the two stable kinds of water. Erik Dahl (1956) has diagrammed this (Fig. 2) and has pointed out that when there is contact with the sea (the left side of his diagram), two unstable environments, brackish and metahaline, may alternate with the two stable types, fresh and marine. What he terms poikilohaline waters alternate with homoiohaline. If the system is cut off from the ocean (the right side of his diagram), there is a continuum, and the whole spectrum, barring fresh water, is poikilohaline. The tolerances of metahaline and polyhaline species overlap. I would eliminate the mesohaline range because there can be no mesohaline species that do not tolerate one or the other of the two stable waters; *i.e.*, the mesohaline range should be combined with the polyhaline or with the oligohaline. If any species (mesohaline) were adapted to tolerate only a narrow range at some salinity other than fresh or marine, it could not persist. The continuum shown by Dahl (1956) makes it clear why methahaline organisms are also brackish; being adapted to hypersalinity without ability to withstand flooding by freshwater or by marine water would be an adaptation of no value.

I have worked with brines in which there are organisms capable of living at 300 ppt. Relative to the tolerances of *Artemia* and *Dunaliella*, for example, tolerance of hypersaline organisms to 80 ppt seems hardly noteworthy. And I work with plankton, including single-celled algae, especially diatoms. The salinity tolerances of single cells is worthy of comment. Steeman-Nielsen, who is concerned with productivity of single-celled planktonic algae, is quoted by Segerstråle (1957) to the effect that attempts to classify waters into categories based on salinity are meaningless. This is because many protists, including single-celled algae, are euryhaline. I once compiled a list of 35 to 50 species of holeuryhaline diatoms—species indifferent to salt concentrations from freshwater to hypersaline. I have no doubt the same could be done for protozoa.

What happens when we shift to metazoa is illustrated by Figure 1, taken from Kinne (1963) which shows the ability or inability of certain crustaceans to regulate their blood concentration. The two species shown by dotted line are hyperregulators: their blood concentration is above (hyper) that of the medium at low concentrations. They tend to be iso-osmotic at higher salinities of the medium. All the species shown in Figure 1 are euryhaline; but two, the decapod *Rhithropanopeus* and the amphipod *Gammarus deubeni*, regulate effectively only below 20 ppt. Lack of regulation at 35 ppt does not mean that they do not thrive in sea water. They do. The other three species shown in Figure 1, the fiddler crab *Uca*, the shore shrimp *Crangon* and the brine shrimp *Artemia* are called hyper- hyporegulators. They do maintain a fairly constant internal milieu; they regulate both at reduced and at increased salinity. So, to repeat, five species are shown in the figure; all are euryhaline, three regulate, two do not. Physiologically they differ, ecologically they are similar.

The regulation curve for *G. deubeni* is quite similar to that of a stenohaline freshwater crab, *Potamon*, shown in Figure 3. *Potamon* is no less an efficient regulator of its body fluid than is *G. deubeni*; but *Potamon* is limited to fresh water whereas *G. deubeni* thrives in both fresh and sea water. And if we compare *Potamon* with *Eriocheir*, the Chinese mitten crab, we find both regulate well in freshwater and are poor regulators at concentrations above 20 ppt. Yet one, *Eriocheir,* is holeuryhaline and thrives at all salinities; the other, *Potamon,* dies in anything but freshwater.

If we consider the curve for *Potamon,* we find the generalization of Claude Bernard is verified: the constancy of the internal environment is maintained in the range from 0–20 ppt, and the animal lives only within the range in which it maintains the constancy of its internal milieu. But *Eriocheir* thrives despite internal inconstancy. And one cannot generalize about holeuryhaline species because some, such as *Artemia,* are hyper-hypo-regulators which *do* maintain their internal fluid constant.

These physiological aspects serve to revive an idea expressed by J. A. Ramsay (1954) at a symposium on active transport. He pointed out that an animal can get along at salinities other than that of its interior either by evolving good powers of active transport in all body cells or by evolving an internal medium which is kept constant by active transport of a few cells of its body, those which separate the blood from the external medium, *e.g.,* gills or gut. The second method is the

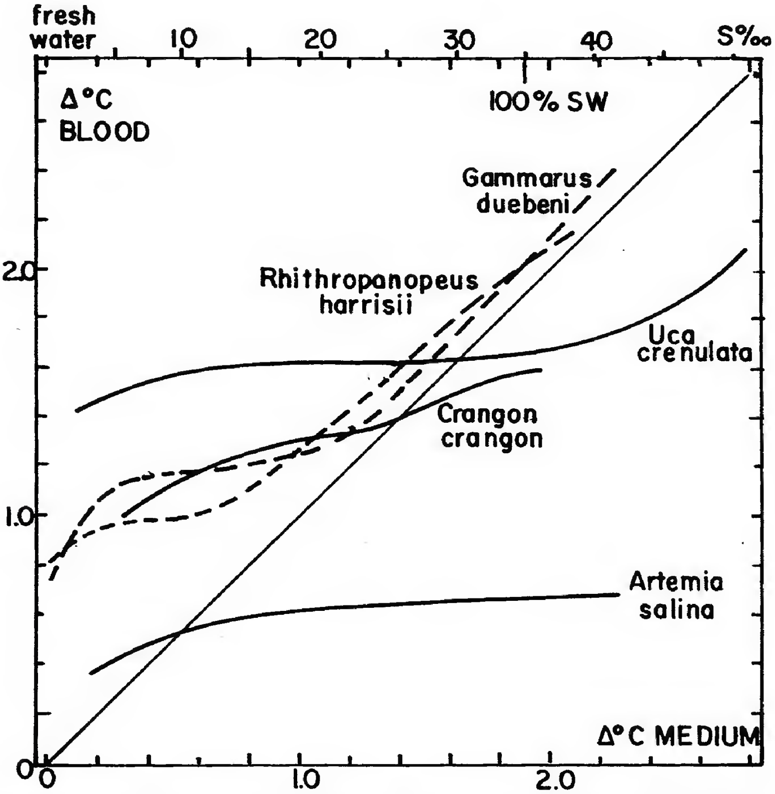

FIG. 1. Blood-osmoconcentration as function of salinity in euryhaline Crustacea. Hyperosmotic regulators: amphipod *Gammarus* (from Kinne 1952) and decapod crab *Rhithropanopeus* (Kinne and Rotthauwe 1952). Hyperhypo-osmotic regulators: decapod land crab *Uca* (Jones 1941), shore shrimp *Crangon* (Flugel 1959), and anostracan brine shrimp *Artemia* (Croghan 1958) After Kinne 1963.

more efficient, but it cannot be used to the exclusion of the first because cells are not identical and the internal medium cannot be in equilibrium with them all. In metazoa some work has to be done by the cell membrane; in single celled organisms such as diatoms the cell membrane must do all the work.

In tracing the development of ideas on the subject of active transport, Ramsay (1954) said that Claude Bernard's proposition, that the fixity of the internal medium is essential for life, has been too well-accepted. Taken literally, this dictum of a vertebrate physiologist would rule out the possibility that an organism without a circulatory system could exist at all, much less penetrate into freshwater or into hypersaline water from a supposedly marine origin. Ramsay states, "We have been too ready to believe that once the internal medium is stabilized, all is over, bar the shouting. We have concentrated too much on the active transport mechanism at the surface of the body and in the excretory organ. We have been inclined to think of the cells of the body as being able to relax, as it were, in a medium with which they are in equilibrium. And this in spite of the abundance of evidence which shows that the cells of the body are not in equilibrium with the fluid which bathes them, but are actively taking up some materials and are actively keeping others out."

Blood can be of secondary concern. Single cells can live successfully in brines, which to a single cell are comparable to blood. In marine and freshwaters single cells are in a relatively constant medium; in brackish and hypersaline waters they are in a medium not constant in many ways. The graph (Fig. 3), showing the relation of internal and external medit for *Eriocheir*, illustrates that at least in this metazoan, euryhalinity is possible without constancy of the cellular environ-

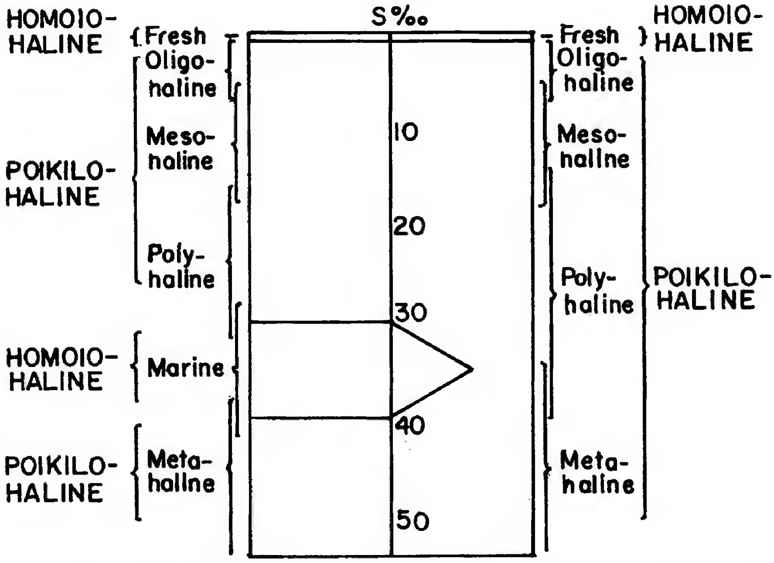

Fig. 2. A diagrammatical representation of the relationship between poikilohaline and homoiohaline bodies of water as well as of the ecological subdivision of poikilohaline waters with respect to salinity. The left half of the diagram represents a poikilohaline system in free communication with the ocean, the right half shows the effect of a decreasing interchange between the ocean and the poikilohaline body of water. (After Dahl 1956.)

ment. Evidently some metazoa have retained cell regulation. Others seem never to have had it or to have lost the ability.

Marcel Florkin (1960) says that to a comparative biochemist each organism is characterized by its own biochemical differentiation on the cellular level and by its pattern of metabolic integration. He elaborates that there are so many interrelations and means of integration, that there is no unique adaptive solution to a biochemical problem such as osmo- and ionic regulation. To paraphrase Florkin, we should not expect to find a single generalization applicable to all organisms, especially at present when there has been no systematic study and there is near absence of genetical knowledge.

Florkin points out that some crustacea when placed at other than sea water salinity have an increased oxygen consumption, which is taken to represent energy expenditure necessary for active transport. Others, *Artemia* for example, exhibit no perceptible change in metabolic rate when in high salinities. Some freshwater organisms actually use less oxygen at low salinities than at higher concentrations which are iso-osmotic to their body fluids. Some organisms, then, seem

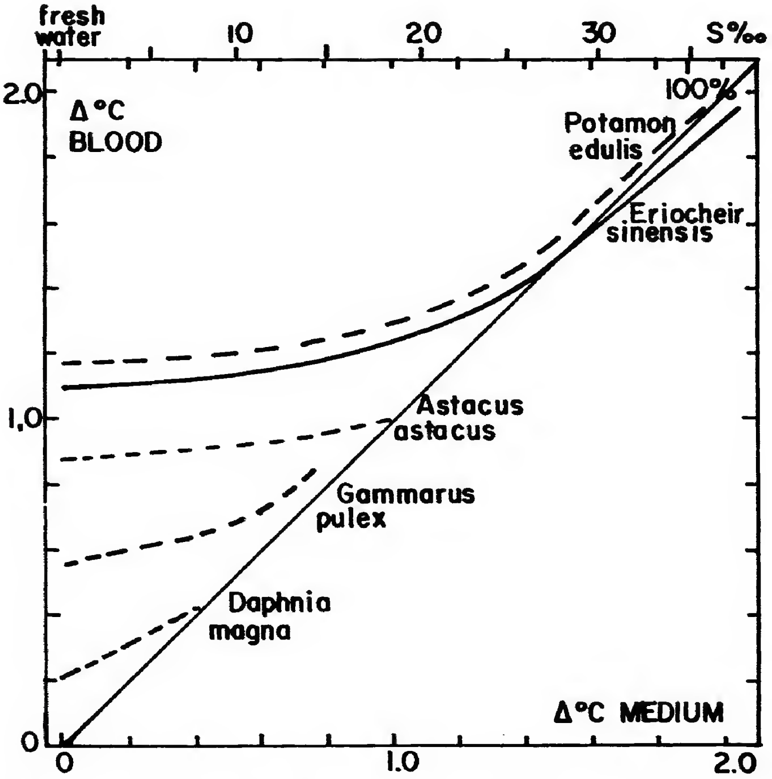

FIG. 3. Blood-osmoconcentration as function of salinity in oligostenohaline and holeuryhaline Crustacea. Oligostenohaline inhabitants of freshwater: decapods, river crab *Potamon* (from Duval 1925; Schlieper and Herrmann 1930) and crayfish *Astacus* (Herrmann 1931; Beadle 1943), amphipod *Gammarus* (Beadle and Cragg 1940), and diplostracan water flea Daphnia (Fritzsche 1917). Holeuryhaline inhabitant of sea, brackish, and fresh water: decapod crab *Eriocheir* (Scholles 1933). (After Kinne 1963)

to have an effortless tolerance when they should in theory be under omsotic stress. Florkin mentions that in the euryhaline crabs, *Eriocheir* and *Carcinus*, there are changes in amino acid content of muscles, there being high concentrations in high salinity and less amino acid at low salinity. Glycine and proline are the two especially involved. Granted that these are not a proven factor in resistance to osmotic stress, the use of organic acids to offset inorganic ions outside the cell is not implausible. The amino acids and short-chain proteins are also of interest in respect to heat tolerance, being more stable at high temperatures than complex proteins. This is mentioned here because holeuryhaline diatoms are also among the most heat-resistant of all organisms. It is commonplace that tolerance to heat and to salinity may go hand in hand.

Let us return to the observation that there may be no increase in oxygen consumption under osmotic stress. Maybe it is just difficult to measure. Potts (1954) quoted in Robertson (1960) calculated that the total osmotic work required for the crayfish, *Astacus*, to maintain its blood at a freezing point depression (FPD) of 0.9 to 1.0 C amounted to only 0.4% of the total metabolic energy of the animal. In *Eriocheir*, whose blood is at a FPD of 1.1 to 1.2 C, it amounted to 1.3% of the total energy. Small wonder that no increase in respiration is often reported; the amount of work involved in maintaining a difference of one or two osmols is not great. If *Uca* and *Artemia* can tolerate the range from 0–50 ppt, as do many single-celled forms and several insects, rotifers and mulluscs (Hedgpeth [1959] lists 19 orders of animals with tolerance in the hypersaline range of which only four have members able to withstand salinities above 80 ppt), why cannot all aquatic organisms tolerate salinity changes? Robertson (1960) suggests a possible reason. Some crabs show a large increase in oxygen consumption at low salinity. This is an increase on the order of 20% rather than the 1–2% mentioned earlier. In the species with higher oxygen consumption there is evidence of great hydration of muscle at low salinity, and supposedly a decreased efficiency of the hydrated cells. We could postulate a similar situation at higher salinity and an increase in the need for oxygen by dehydrated cells. Species with cells unable to regulate require a constancy of the internal medium. Species with the ability to regulate at the cellular level can get along as *Eriocheir* does, without constancy of the body fluid.

EVOLUTIONARY IMPLICATIONS

There are evolutionary implications to the above discussion. Hedgpeth (1957) in speaking of animals in estuaries said, "Euryhalinity is probably a physiological trait not easily come by and once attained is very conservative." Later on he said, "Euryhalinity then may be a physiological characteristic not of a species or of a genus but of a phyletic stock." The physiological evidence favors the idea that euryhaline organisms are conservative with a long evolutionary history. Vinogradov (1959) discusses the shallow seas at the time of the first appearance of the broad platforms of the continents. There was a time of great $CaCO_3$ deposition, presumably by precipitation from shallow evaporating seas. I mentioned previously the shallowness of the hypersaline lagoons and loss of $CaCO_3$ at the concentrations taken as the upper limit of hypersalinity. The pre-Cambian $CaCO_3$

deposits occurred about 2.4×10^9 years ago, at the period postulated as that of the origin of life. The earth may have been warmer when life began, and hypersaline waters and hot springs are warmer than the present ocean, and in them $CaCO_3$ precipitates at 70 ppt. Shallow waters in the hypersaline range may have been the original home of cellular life.

Kinne (1963) says that hyporegulation . . . "is always correlated with hyper regulation, suggesting that hyper- hypo-regulation is indicative of an advanced stage of genetic adaptation to fluctuating or extreme salinities." He adds that this ability in salt tolerant insects "represents presumably the most elaborate genetic adaptation to osmoic stress within the invertebrates." This does, indeed, seem to be true for body fluid regulation. But what about *Eriocheir*, which is holeuryhaline but without the highly evolved hyper- hypo-regulation? Is it primitive? It would seem that body fluid regulation is superimposed on cellular regulation.

Inside their membranes, the first organisms obviously had the ability to maintain differences from the outside. Active transport is an inherent property of any biological system. A living cell is not an osmometer; it absorbs ions and molecules differentially, and can do so against concentration gradients. Most modern freshwater organisms die in seawater when they fail to make necessary adjustments at the cellular level in response to increases in blood concentration. For example, the crayfish, *Astacus*, exhibits some increase in amino acid concentration when its blood concentration is raised, but not enough to allow it to live in sea water. *Eriocheir* evidently can add enough amino acids and the stimulus for the change seems to be a change in concentration of intracellular sodium, according to a summary by Lockwood (1963). Stephens (1964) finds that *Nereis succinea*, more euryhaline than *N. limnicola*, takes up glycine from dilute solution more rapidly than the latter. These euryhaline worms remove glycine from solution better at intermediate and high salinities than in freshwater.

The most likely answer to the question raised by Hedgpeth regarding euryhalinity as a property of phyletic stocks is the idea of N. W. Pirie (1959), expressed in another of the papers in the Moscow Symposium. When life began there was morphological simplicity (cell-like ?) but greater chemical diversity than there is now. The present similarity of all metabolic functions is the result of selection of the most efficient or best-adapted reactions. But as a result of the original diversity, it is possible for continuation of phyletic differences and for continuation of some once-important capacities by various lines. Andre Lwoff (1943) has called attention to the evidence that much of evolution is accompanied by selective loss rather than by gain in capacity. In homoiohaline waters there has been no disadvantage to losing the ability to regulate at the cellular level. Loss is no handicap, but it means exclusion from poikilohaline waters unless there has been the acquisition of ability to regulate at the body fluid, or at the organismal, level.

Suggested here is that cellular regulation is an old and conservative feature, that body fluid regulation is new and is superimposed on the old. The two levels of regulation may be present in various combinations in different species.

There are relatively few euryhaline species because there is a relatively small amount of poikilohaline water. But the unstable waters have always been present and they allow the continued existence of ancient cellular capacities as well as having allowed new adaptations for existence in the fluctuating environment provided by brackish and hypersaline waters.

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VERTEBRATES IN HYPERSALINE WATERS

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ABSTRACT

Marine hypersaline areas form on dry coasts in lagoons and bays that have some connection with the open ocean. Marine mammals and reptiles are few in contrast with the abundance of fish-eating birds. The fishes invading supersaline areas are euryhaline estuarine species, which seem to become more easily adapted to hypersaline conditions than fishes which live in the more constant salinities of seawater. Occasionally extreme conditions exist when the salinities reach an equivalent of about 100 ppt and all animals die. Populations are re-established by re-invasion from nearby estuarine waters. Hypersaline lagoons used for the effluent of plants making fresh water from salt water would have to be quite large in order to maintain fairly stable conditions if fish were to live in them. Large shallow flats in some parts of the world become hypersaline with small scattered pools and have low fish productivity. These sometimes have large numbers of insect larvae and other invertebrates. If such flat areas are available for the disposition of effluent, they might be more economical than lagoons.

INTRODUCTION

The average salinity of the large oceans of the world varies from about 32 to 36 ppt salt, but there are two seas of considerable size, the Red Sea and the Persian Gulf, in which the salinities rise to a little above 40 ppt. For that reason I believe that hypersaline waters should start at about 45 ppt. Zernov (1949) in his book on General Hydrobiology has arrived at 47 ppt as the lower limit of hypersaline lagoons.

Most large hypersaline water bodies of the world are of fresh water origin. The largest ones are the Great Salt Lake of Utah, the Dead Sea and the Gulf of Karabugaz (Andrussov 1897) on the eastern shore of the Caspian Sea. There are many similar but smaller water bodies scattered over the face of the Earth. The chemistry of these waters is variable, but they all have one characteristic in common. They are so salty or so high in the so-called alkali salts[1] that they support no vertebrate life. For that reason the hypersaline non-marine waters can be ignored for the purposes of this paper, and largely they can be ignored for the purposes of this symposium because these waters lie in arid regions where water suitable for desalinization is not present. The Gulf of Karabugaz is an exception and there may be others. With salinities ranging from 163 to 285 ppt, it lies close to the Caspian Sea which has a salinity of around 12.8 ppt (Zenkevich 1957).

The exclusion of the fresh water brines and alkali waters from consideration leaves the hypersaline water bodies along the margins of the seas to be examined. These are several kinds. The largest and most stable are the lagoons.

In Bulgaria there are some hypersaline seaside areas called limans, which have been described by Caspers (1952). These are small and artificially controlled areas for the making of salt. In Egypt there is the Bitter Lakes area, but these are relict fresh water brines which have become freshened by connection with the Suez Canal. In Africa the Santa Lucia Estuary of the Zululand coast is hypersaline. It has only recently been blocked off from the sea and the high salinity seems to be derived from inland briny lakes. The hypersaline water has covered the whole estuary now that the sea has been excluded. The area has been studied by Day *et al.* (1954).

In the following pages, some information about vertebrates in natural marine hypersaline waters will be given, with the hope that it will lead to an understanding of what might happen in man-made hypersaline lagoons and ponds created as a result of desalinization.

HYPERSALINE LAGOONS

As basins lying along the margins of the seas, the hypersaline lagoons are long, narrow and shallow. They develop only in arid regions where they receive very little fresh water inflow. Embayments are minor because of the lack of river flow, which explains in part the great length and small width. These lagoons are separated from the sea by peninsulas or islands with only narrow connecting openings, which are a *sine qua non* for the development of hypersaline lagoons. Water flows in from the sea and evaporates, leading to hypersalinity. If openings to the sea were large enough to permit sufficient circulation, the lagoons would be only arms of the sea with salinities comparable to it.

There is only one hypersaline lagoonal area in North America, the Laguna Madre of south Texas and the Laguna Madre del San Antonio of northern Mexico, also known recently as Laguna Tamaulipas. This is virtually a continuous system covering some 250 miles of coastline, but it is separated in the middle by 15 miles of sea coast and the Rio Grande which flows directly into the sea.

A similar lagoon, the Sivash, lies next to the Sea of Azov in the Crimea. The most extensive account of the Sivash in English is given by Hedgpeth (1957).

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[1] The alkali salts of the American West, famed in fact and fiction as poisoning the waters of that region, are the sulfates, carbonates, bicarbonates, borates and nitrates of sodium, potassium, magnesium and calcium.

Hypersaline lagoons are quite uncommon along sea coasts and I know of no others than those mentioned above, but there may be some other small ones along arid, isolated coasts of Asia and western South America.

THE SIVASH

This body of water is about 70 miles long and its widest part is about 15 miles, but it is intersected by paired and unpaired bars or spits and areas of open water are reduced. These spits also, no doubt, interfere with circulation. This lagoon connects by one narrow channel with the Sea of Azov, which in turn connects with the Black Sea. The Black Sea salinity runs about 17.5 ppt while that of the Sea of Azov is about 11 ppt. Obviously the high salinity of the Sivash is caused by low rate of mixing with waters of its parent body.

The greatest depth in the Sivash is 3.2 m (10.4 ft.). Summer temperatures of 30–35 C are found and the water freezes over in the winter. The salinities range from 36 ppt near the inlet channel to 132 ppt at the lower end. Certain worms and molluscs extend southward to the closed end. The average yearly fish catch of the Sivash has been reported to be 80–90 tons, although one author has said that it would be 21,000 tons if all the plants produced in the area were consumed. The commercial catch of mullet (*Mugil cephalus*) sometimes equals 500 tons (one million pounds) a year. Other fishes that enter the highly saline waters are flounder, *Pleuronectes*, and a goby, *Zosterioessor*. The mullet is reported to enter waters up to 113 ppt.

This area has high salinity kills, of course, and in winter it has cold kills. As Hedgpeth (*op. cit.* p. 713) said it is "remarkably similar in some of its physiographic and biological characteristics to the lagoons on the coast of Texas and northeastern Mexico."

THE LAGUNA MADRE OF TEXAS

The Laguna Madre is 130 miles long and extends from Corpus Christi, Texas to the Mexican Border. It is three to five miles wide and no deeper than 10 feet anywhere. A great part is sand flats a foot or less deep. In the middle region there is a sand flat extending across the Laguna which is dry most of the time, so that essentially the Laguna is now two bodies of water. A tributary, Baffin Bay, with the same general characteristics, joins the Laguna Madre about one-third of its length from Corpus Christi Bay (see Figure 1). The whole region lies in a rainfall zone which Thornthwaite (1948) has classified as semi-arid.

This region has had water temperatures up to 44 C recorded over the shallow flats (Simmons 1957).

In the winter the hard cold waves that strike the Texas coast about every 10 years (Gunter 1940) kill more fish in the Laguna Madre than anywhere else on the Texas coast, although it is farther south. This is because the Laguna becomes colder than the waters 50 or 60 miles to the north. In the hardest cold spells on the Texas coast I have never found the brackish bays with an open water surface temperature lower than 4 C, even when there was mush ice on shore. However, Mr. Ernest G. Simmons, of the Texas Parks and Wildlife Commission, pointed out to me not long ago that the Laguna water drops to zero C. The Laguna is shallower on the whole than the other bays and it would tend to become colder at equal temperatures, but it does not freeze because of the high salinity. Thus the thermostatic effect of water turning into ice does not operate, according to the principle that one cubic centimeter of water turning to ice gives off 80 calories of heat. So one extreme of a harsh environment accentuates another in this case.

Catastrophic salinity kills formerly occurred in the Laguna Madre about every ten years before the 1940's. These catastrophic mortalities along the south Texas coast are of significance both to fisheries biology and paleontology (Gunter, 1947; 1952). The last bad fish-kill in the Laguna occurred in the late thirties. Small ones occur every year. but the large ones kill out all the fishes and apparently the salinities run from 80 to 110 ppt and possibly more.

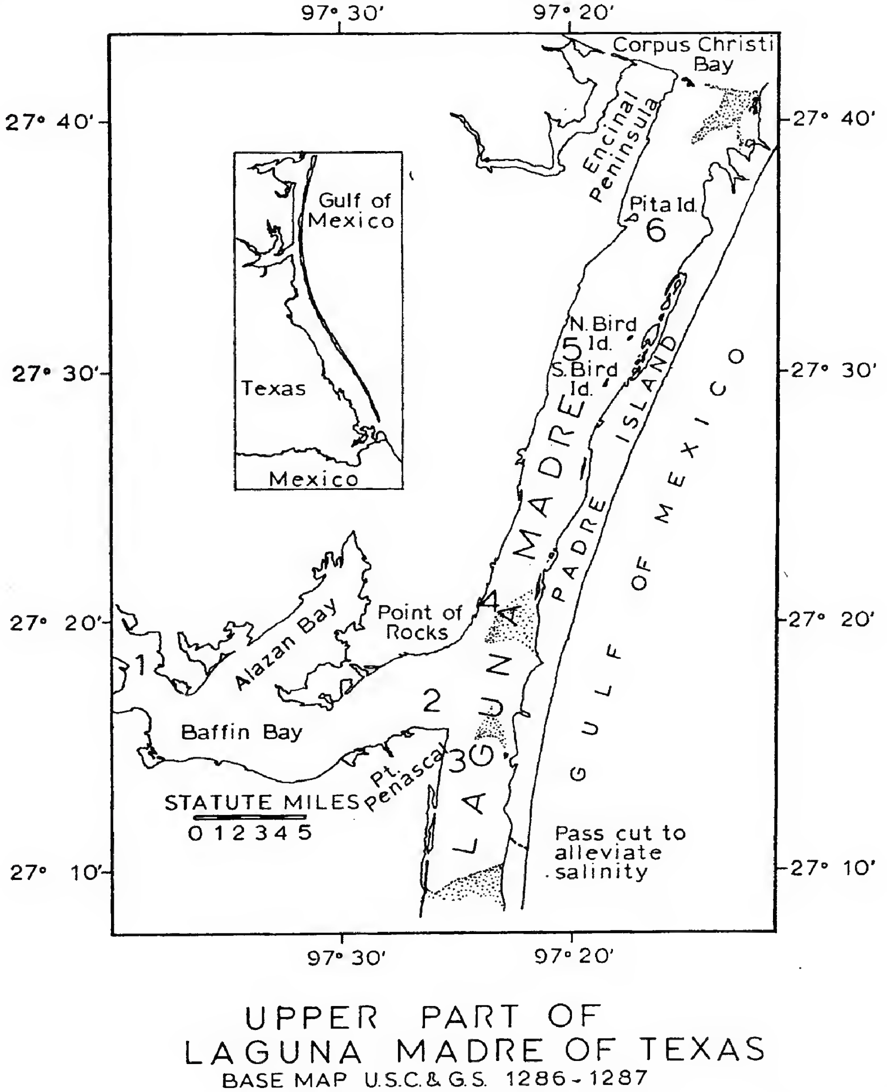

Fig. 1. This map was modified from Hedgpeth (1947). The whole Laguna plus Baffin, Alazan, Corpus Christi and Nueces bays are shown in the inset.

Attempts were made in the 1940's by the Texas Game, Fish and Oyster Commission to cut passes to the Gulf to alleviate the high salinity, but the passes were so small as to be useless, and they soon filled. It was pointed out (Gunter 1945b) that the size of the channels dug through Padre Island to the Gulf was related to the Laguna as a pencil mark would be to a shallow ditch 130 feet long and five feet wide.

Then an unforeseen effect of the Intracoastal Waterway solved the hypersalinity problem of the Laguna Madre. When the Waterway was extended down the Laguna from Corpus Christi Bay to Brownsville in 1948 it was found that a head of water existed between Corpus Christi Bay and the lower Laguna, causing a flow southward which flushes the hypersaline waters.

The studies of Breuer (1957) and Simmons (1957) were made after the Waterway completion and it is clear that the Laguna Madre is still hypersaline. But it no longer becomes a deadly brine, and it did not do so during the most severe drought Texas has ever experienced, which lasted for ten years and was broken in 1957.

VERTEBRATE SPECIES IN HIGH SALINITY WATER

IN HYPERSALINE COASTAL LAGOONS

The only common marine mammal in south Texas is the bottlenosed dolphin, *Tursiops truncatus*, but it is not found in the Laguna Madre. Probably the briny solution causes stinging sensations to the eyes and other parts of porpoises, and they stay out of high salinity areas. It is known that fishes in the Laguna often go blind during years of high salinity and their eyes take on a "cooked" appearance.

At the other end of the chordate array, the acorn worms, *Balanoglossus*, Hemichorda, the Tunicates (Urochorda) and the Acrania (Amphioxus) have not been recorded in the Laguna. Representatives of all these groups are found in the nearby bay waters. Apparently they shun the hypersaline waters. There are no cyclostomes in the shallow Gulf. In short, the vertebrate residents of the Laguna Madre are the elasmobranch and teleost fishes.

There is a well-known principle of biogeography which states that impoverishment of species takes place as the physical factors of the environment vary from the optimum. This fact is so old and well-established that its origin is lost, but Edward Forbes, who is generally considered to be the father of marine ecology, noted the diminishing effect of temperature on the numbers of species towards the poles (Forbes and Godwin-Austen 1859). The German zoogeographer Richard Hesse treated this subject in some detail, and gave examples of decline of species number of marine animals with depth, temperature decline, and both decrease and increase of salinity from the optimum, which is undiluted sea water (Hesse, Allee and Schmidt 1947).

A corollary of this principle is that the numbers of a few species may increase enormously in numbers. Parker (1959, p. 2158) has refined this principle with regard to invertebrates as follows:

> "Both in hypersaline and very low-salinity regions, the variability and adversity of the environment determine the species composition and com-

> parative abundance of each species. In extremely variable hypersaline areas, the number of species is very low, and the number of living individuals is small also. In stable hypersaline areas (and stable, very low-salinity waters), the number of species is still small, but the number of living individuals is extremely large. As the salinity decreases or increases to normal values (along with relative stability), the number of species increases and the number of individuals per species decreases."

There are indications that Parker's principle also applies to the fishes.

The hypersaline Laguna Madre illustrates well the impoverishment of fish species with the high salinities. The data are taken from the three papers of Gunter (1945a), Breuer (1957) and Simmons (1957).

In the normal, low salinity bays, Copano and Aransas, fifty miles north of the Laguna Madre, Gunter found five elasmobranchs and 82 teleosts in a two year survey. Simmons found six elasmobranchs in the Laguna and 67 teleosts, but his area of study extended from the Corpus Christi Bay entrance and included areas of normal sea water salinities, the range being 36.1 to 80.0 ppt. Simmons' remarks under individual species indicate that only one elasmobranch and 45 teleosts were taken in the hypersaline waters of the Laguna (above 45 ppt). Breuer (1957) found one elasmobranch and 24 teleosts in Baffin Bay and its connections. The salinities at Breuer's stations ranged from 1.40 to 75.05 ppt and averaged 51.7 ppt. This inner part of the hypersaline area has the highest salinities of all on the average, but stations near the dry arroyo mouths sometimes have low salinities following the infrequent rains.

Simmons (1957) listed the fishes normally collected in salinities up to 75.0 ppt in the Laguna and Breuer listed the fishes "which can be considered common residents of" Baffin Bay. The comparison, while not strictly comparable, is close. The fishes are listed in Table 1.

Simmons' table 8 shows that 67 species were taken in water up to 35 ppt, 52 up to 45 ppt, 19 up to 60 ppt and 10 up to 75 ppt. He noted that small speckled trout were taken in waters up to 60 ppt salinity, but the adults were commonly

TABLE 1

Fishes normally caught in hypersaline areas in the Laguna and Baffin Bay. From Simmons (1957) and Brewer (1957

Laguna Madre To salinity 75 ppt	Baffin Bay Includes some species found only to 50-60 ppt
Elops saurus	*Galeichthys felis*
Anchoa hepsetus	*Cyprinodon variegatus*
Fundulus similis	*Menidia beryllina*
Cyprinodon variegatus	*Mugil cephalus*
Menidia beryllina	*Lagodon rhomboides*
Mugil cephalus	*Cynoscion nebulosus*
Lagodon rhomboides	*Sciaenops ocellatus*
Cynoscion nebulosus	*Micropogon undulatus*
Pogonias cromis	*Pogonias cromis*
Micropogon undulatus	*Paralichthys lethostigma*

found up to 75 ppt. This is an unexpected verification of the fact I have pointed out many times, namely that the larger animals of a species are generally found in the higher salinities and the smaller animals in the lower salinities (*Cf.* Gunter 1961).

Simmons concluded with regard to salinity that in normal ranges of hypersalinity (below the range of salinity kills) the higher the salinity (1) the fewer the species, (2) the greater the number of individuals of each species, (3) the larger the average individual of each vertebrate species and (4) the smaller the average size of several invertebrate species, such as barnacles and the blue crab (*Callinectes*). Stunting in high salinity waters has been reported many times for invertebrates and it has been considered extensively by paleontologists (*Cf.* Hallam 1965), but it is outside our purview and apparently does not occur in hypersaline vertebrates.

Simmons also found that the range of 40–50 ppt seemed to be the optimum for many species, and 21 animals became less abundant when waters fell below 40 ppt.

Simmons noted that in general high temperature and high salinities in the Laguna coincided seasonally and that when high salinities coincided with low temperatures "many forms not normally tolerant of hpyersalinity were present." These included certain invertebrates and the redfish, *Sciaenops*, and the flounder, *Paralichthys.* On the other hand certain bivalves tolerated extremely high temperatures at moderate salinities. There seems to be an oxygen problem involved here, as was first pointed out by the late Colonel J. G. Burr, of the Texas Game, Fish and Oyster Commission. The dissolved oxygen content of the water decreases, of course, with high temperature and also with the increase in salinity.

Old commercial fishermen on Baffin Bay have told the writer that even when the salinities were very high that so long as the wind was blowing and kicking up some waves (and it is very windy country, average 11 mph all the time) the fish were all right, but when it got still and the water became glassy the fish came to the top gasping and the fish-kills began. Thus the fish-kills in hypersaline waters may be as much connected with oxygen lack as osmotic disturbance. Of course the two are connected for it takes considerable work for a fish to regulate its fluid concentrations, where the osmotic pressure may be 30 atmospheres different internally and externally.

Occasionally such high seas fishes as the bluefish, *Pomatomus*, and the Spanish mackerel are taken in the lower salinity areas of the Laguna. Even so, these strictly marine species are absent from the high salinity Laguna, although there is no barrier to keep them out at either end. As Hedgpeth (1957) has noted before, the fauna of hypersaline coastal lagoons is derived from nearby brackish waters rather than the sea. The organisms which live at hypersalinities in The Sivash come from the Sea of Azov which has a salinity of about 11.0 ppt. Similarly, every fish taken in the Laguna Madre has been commonly recorded in the low salinity, connecting bays to the north.

It should be noted also that every species of fish listed in Table 1 is also on the lists of euryhaline fishes of North America (Gunter 1942; 1956), which is to say they have been recorded under natural conditions from fresh water and also full sea water. It would seem that fish which are required to carry on osmoregulation in waters of varying salinity are better able to cope with hypersaline waters than those species that live in the higher but unvarying salinities of the high seas. In this connection it should be noted that the writer (Gunter 1945a) once took *Cyprinodon variegatus* and *Menidia beryllina* in seines at Caroline Beach on Baffin Bay at a salinity of 71.5 ppt. Seventy-nine days later, following cloudburst rains, the two fishes were still present at a salinity of 2.2 ppt.

The Laguna Madre has been famous for its fish production and Hedgpeth (1947) has shown that from 1942–46 inclusive it furnished 50% of the commercial fish production of Texas, although it equals only 20% of the bay area. The commercial fishes caught in great abundance are all croakers or drums, family Sciaenidae. They are the redfish, *Sciaenops ocellata*, the black drum, *Pogonias cromis*, and the speckled trout or weakfish, *Cynoscion nebulosus*. The abundant mullet are not commercial fish in Texas. There are other more typically Texan testimonials of the abundance of fishes in the Laguna.

The Laguna Madre is surrounded by wild, desolate country and a great part of it is bordered by the King and Kennedy ranches, which permit no entries. Therefore the Laguna Madre can be entered only by boat at the upper and lower ends. It is full of fish but sportsmen have had it closed to commercial fishing for several years. As I have pointed out before (Gunter 1964) the laws against commercial fishing in Texas bays have caused the loss of a quarter of a billion pounds of foodfish in the past forty years and they have conserved nothing.

The Laguna Madre is virtually a closed system so far as nutrients are concerned, but none the less, a productive one so far as utilizable fishes go. The basic productivity of the Laguna has been actively studied by staff members of The University of Texas Marine Science Institute at Port Aransas and we may look forward to seeing the basic factors of the productivity of these waters clearly delineated some day.

TIDE POOLS AND SHORE PONDS

There are several kinds of shore ponds and pools, rock pools, ponds in loose sands or in more impervious substrata. They may be renewed and refilled every day on some coasts by normal tides, or they may be caused by hurricane tides which come many years apart. In the latter instances the ponds may gradually dry up and become hypersaline.

Only ponds in arid or semi-arid climates become hypersaline. Such ponds may form on bay shores, as well as on the seashore. The fate of these ponds in general is to achieve new connections with the sea during high tides or to dry up. A few may remain for years with sufficient fresh water inflow or rainfall to sustain them until the shore topography changes. As such they do not become hypersaline. As a whole they are transient.

Animals entering the seaside ponds are of course marine. The fishes seem to die rather quickly and the chief fish found on the Texas coast has been *Menidia beryllina*. Sometimes, young tarpon are found in such ponds at salinities of 50 ppt.

In 1950–51, Simpson and Gunter (1956) made a study of the cyprinodontids of the Texas coast. Many ponds and sloughs along the bay shores were visited between Trinity Bay (East Texas) and the Rio Grande. Even in mud puddles we found no salinities above normal sea water except in the subhumid and semiarid zones. Sloughs with intermittent bay connections had the highest salinities. These locations were either barren of fish life or replete with cyprinodontids, usually with *Cyprinodon variegatus* predominating.

Stations at seven ponds and sloughs with salinities ranging from 37.7 to 44.8 ppt yielded 668 *C. variegatus*, 185 *Fundulus grandis*, 68 *F. similis*, 11 *Lucania parva*, 10 *F. pulvereus* and 9 each of *Adinia xenica* and *Mollienesia latipinna*; stations at five ponds and sloughs with a salinity range of 47.6 to 54.4 ppt yielded 834 *C. variegatus*, 34 *F. similis*, 14 *A. xenica*, 9 *F. pulvereus*, 9 *Mollienesia latipinna*, 6 *F. grandis* and one *L. parva*; three stations with a salinity range of 60.9 to 64.9 ppt yielded 1139 *C. variegatus*, 12 *F. grandis* and 4 *F. similis*. One station at 76.1 ppt yielded 259 *C. variegatus*, 16 *F. similis* and 1 *F. grandis*. A station with a salinity of 88.8 ppt gave 795 *C. variegatus* alone. Two stations at 130.8 and 147.2 ppt had no fish, but one with a salinity of 142.4 ppt, a drying pond, yielded 2009 *C. variegatus*. When this last reading was made it was the highest salinity at which a fish had been taken, but that record has since been broken, just where I have forgotten. Neither sun nor snow nor high salinity kills *Cyprinodon variegatus* and I believe it to be the toughest aquatic animal in North America.

Shore ponds and pools are not hypersaline except in dry zones. They are transient and not very important to the subject at hand. The chief fishes found in them are the cyprinodontids.

SALT FLATS AND SALINAS

There is another type of hypersaline environment about which little is said in the ecological literature. These are low-lying areas next to the shore with interlacing channels and ponds, sometimes connected with the sea and sometimes not. One of these flats lies on the Aransas National Wildlife Refuge where the Whooping crane winters (Gunter 1950; Hedgpeth 1950), but the area was not hypersaline and only became so later during an extreme drought (*see* Hoese 1960).

The hypersaline salt flats have been described mostly in connection with studies of the flamingo. In his account of the *Voyage of the Beagle* Darwin said, ". . . . I met with these birds wherever there were lakes of brine." R. P. Allen (1956) stated in his magnificent study of flamingos that they nest in fresh water areas in rare cases, but for reasons unknown do not do well there and are found mostly around salt lakes and salinas of "desolate and dreary conditions." Such an area is the Camargue in southern France, which is now a reserve for the nesting flamingos there; but they are having trouble because rice growers along the Rhone are flushing too much fresh water into the area.

Allen has pointed out that the food of flamingos in the Caribbean region consists mostly of insect larvae, annelids, crustaceans and certain molluscs which are able to withstand the high salinities. He said the only fish eaten in the Bahamas was the local variety of *Cyprinodon variegatus*, that being no surprise for it was probably the only fish present.

I determined the salinities for Allen from twenty-four nesting areas. Eight areas had salinities ranging from 27.5 to 37.3 ppt. Nine areas had ranges of 45.9 to 97.2 ppt, and seven areas had salinities ranging from 119.8 to 183.5 ppt. The latter station was at Bonaire, Dutch West Indies.

According to Rooth (1965), the several different salinas of Bonaire have salinities ranging from 20.3 g chloride per liter to 114.7 g cl/l. This is equivalent to about 37.0 and 206.0 ppt salinity. This author showed that the larvae of *Ephydra*, the brine fly, is almost the total food of the flamingos in this region.

Rooth discusses the topography and hydrography of the salinas of Bonaire extensively. Essentially, they are low flat areas, nearly at sea level or below in some places, separated by low ridges of coral rock from the sea, with only intermittent connections. The low areas are salty ponds or may even be dry.

SUMMARY AND CONCLUSIONS

Hypersaline marine waters occur only along sea coasts in arid or semiarid zones. They are connected to the sea or adjacent bay waters only intermittently or through narrow channels which permit little exchange with larger bodies of water. The salinities range between 45 and 206 ppt.

According to Copeland (1967) ferric oxide and calcium carbonate begin to precipitate out of sea water solutions at just below 70 ppt and various others drop out at higher salinities. Thus the so-called ppt salinity above 70 is not comparable to sea water, and above this figure the ionic environment as well as the osmotic pressure is quite different from sea water.

Hypersaline areas may be divided roughly into three types. One is the long, narrow saline body of water called a lagoon, and another consists of pools and shore ponds, intermittently connected with the sea. The third type is the salt flat or salina, which is an area of low relief, partially or completely blocked off from the sea except at highest tides, with completely dry depressions or briny pools. In the very saltiest situations no macroscopic life exists, but there may be brine flies up to 140 and 150 ppt. A few fishes exist at salinities of 100 ppt and *Cyprinodon variegatus* has been recorded at 142 ppt.

In artificial hypersaline lagoons and other coastal bodies of water the fishes most likely to be found in the south and southeastern United States and eastern Mexico are *C. variegatus*, *Menidia peninsulae,* various fishes of the family Sciaenidae, and mullet, *Mugil cephalus*. There would be some change of species north of the middle Atlantic and on the Pacific coast.

The numbers of different species of fish in very hypersaline ponds or lagoons would be quite small, but the numbers of individuals might be very large, and in some areas the fishes produced might even be of economic importance.

It would seem that, depending upon the topography of the site, brine disposal from desalinization plants could be drained out over wide, low relief flats or impounded in artificial hypersaline lakes near the sea or bay shore with narrow openings to the natural waters. Under such conditions they would do little harm in non-arid regions. There is no indication that brine effluent drained into a large bay in a humid area would cause hypersalinity of a large body of water in the region. In arid zones such as south Texas and northeastern Mexico desalinization effluents would increase the harshness of an already harsh environment.

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PLANTS AND THE SUPERSALINE HABITAT

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ABSTRACT

In assessing the probable effects of brine waste discharges on marine flora, it is important to distinguish between survival capacity and growth. Organisms may survive temporary increases in salinity several times the level that inhibits growth completely. Metabolic processes may proceed at a nearly normal rate even when cell division is strongly inhibited. The capacity of euryhaline littoral algae to survive periodic high salinities may, therefore, not indicate a comparable capacity for growth when salinity is maintained at an elevated level. Sublittoral red algae are intolerant to hypertonic solutions of about 1.5 times sea water. It has been shown that many marine plants are sensitive to as small as a 20% increase in the salinity of sea water.

INTRODUCTION

Water desalination plants may become a major source of highly saline effluents into oceans and seas. The salinity of waste brines from such plants depends on plant design and operation (von Lossberg 1966), and variations in brine-disposal methods will also affect the salinity of the receiving waters (Pritchard, Carter and Okubo 1966).

The probable damaging effects of any system of saline water disposal on littoral and sublittoral species of marine flora can be evaluated by considering the tolerance of plants to any increment of salinity in excess of present or "normal" levels, rather than arbitrarily defining "supersalinity" at a specific limit.

My research experience with land plants (primarily crop plants) supports the desirability of considering the effects of *all* salinity levels rather than those of a restricted range. Land areas present a continuous spectrum of salinity levels and, unlike the oceans, there is no "normal" salinity. Although an arbitrary lower level has been used to separate saline lands from nonsaline (U. S. Salinity Laboratory 1954), in practice this has been found to be more useful in classifying land than in predicting the effects on plants. The most sensitive species are affected even under some nominally nonsaline conditions, whereas salt-tolerant species may produce full yields on nominally saline lands.

On the land, there are wide differences in the composition as well as in the concentration of soluble salts. Specific ion effects are, therefore, a major concern in investigating the salt tolerance of land plants. Some plants (particularly woody forms, such as trees and shrubs) have forgotten their marine origins and have become specifically sensitive to chloride and sodium ions (Bernstein 1965; Bernstein and Hayward 1958). Transpiration, or the evaporative loss of water from plants, exerts a significant influence on the response of plants to salinity. Since the supply of soil-water is limited, the concentration of salts in this water increases as the water is depleted. (Plants absorb water and salts independently, and under saline conditions, very little of the salts present in high concentrations is absorbed, in relation to the volume of water absorbed.) Also, land plants are subject to longer-term fluctuations in salinity, caused by leaching of salts by rain or heavy irrigation and upward salt movement in the soil during dry periods. These complications are, however, not entirely without significance for marine plants even though they are not subject to these salinity fluctuations, because investigation of the effects of fluctuating salinity has taught us much regarding the salt tolerance of plants. We have learned, for example, to distinguish capacity for survival under saline conditions from capacity for growth. The presence of one does not necessarily imply the presence of the other in like degree. Bell pepper plants can tolerate periodic increases of the osmotic pressure of the root medium to 12 atm but fail to grow when the osmotic pressure is maintained above 6 atm (Bernstein and Pearson 1954). Furthermore, tolerance to salinity may vary with the growth of stage of the plant. Some species are especially sensitive during germination, others during the seedling stage, and still others during flowering (Bernstein 1964; Bernstein and Hayward 1958). These effects have had to be studied in some detail because we must account for salt tolerance during all stages of growth in an environment of fluctuating salinity.

CRITERIA FOR ASSESSING SALT TOLERANCE

Survival—The salinity range that permits the survival of a plant species has been of primary interest to ecologists. This is one of the major parameters governing distribution of salt-marsh species (Chapman 1960; Penfound and Hathaway 1938), although other factors such as frequency and duration of flooding, depth to water table, and soil characteristics are also important. Fluctuations of salinity in marshes are determined largely by frequency of tidal flooding and rainfall. The seasonal salinity range for a given marsh species may be fivefold or greater (Chapman 1960). Desert and arid-zone species are also subject to large seasonal fluctuations in salinity (Harris *et al.* 1924). Although the distribution of species may be more or less determined by the range of salinity, it should be noted that periods of low salinity occur more or less regularly, and that growth may be highly dependent on such periods of minimum stress. Such growth relationships have been rarely measured in ecological work, but some pertinent results from controlled laboratory experiments will be described in the following sections.

Growth—The relationship of growth to salinity, which may be accurately determined under controlled conditions, has been of primary interest to agronomists (Bernstein 1964), but it is also of great significance to the plant ecologist. A plant may tolerate periods of high salinity, but it may make virtually no growth until the salinity is reduced by rainfall or other leaching action. Mere survival limits may, therefore, be misleading in determining the permissible salinity range for continued growth and reproduction. Some growth-response curves for species of contrasting salt tolerance are presented in Figure 1. Relative growth yields, such as are given in Figure 1, are often useful in comparing growth and yield responses of species with greatly different growth potentials (Bernstein 1964). Obligate land halophytes such as *Salicornia* (Chapman 1960) and*Allenrolfea* (U.S. Salinity Laboratory, unpubl.) fail to grow in nonsaline media but grow well over a wide range of salinity with very little effect of increasing salinity on growth. Facultative halophytes, such as *Atriplex vesicaria* (Black 1960), are stimulated at low salinity (approximately 1 ppt NaC1) and show progressive decrease in growth as salinity increases. Glycophytes such as corn (*Zea mays*) show maximum yields under nonsaline conditions and a more or less progressive decrease in growth with increasing salinity. The more tolerant plants in this group have a plateau of growth extending from the nonsaline condition to low or moderate salinity (up to about 5 or 10 ppt in the soil-water) before the yield decline becomes evident at higher salinity (Bernstein 1964).

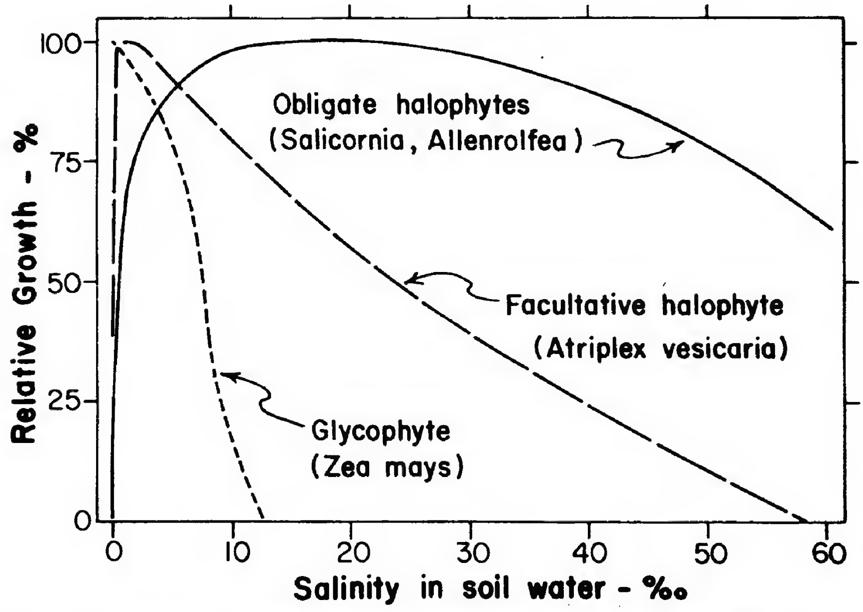

FIG. 1. Relative growth of plants in relation to soil salinity. Modified from Montfort and Brandrup (1926).

SALT TOLERANCE OF MARINE ALGAE

Useful reviews of the literature on salinity and algae are found in "Physiology and Biochemistry of Algae" (*See* Guillard 1962; and Biebl 1962). Major interest has centered in the estuarine and intertidal zones because these are subject to wide fluctuations in salinity, and the plants must, therefore, be capable of rapid adjustment over a wide range of salinity.

In estuaries and tidal inlets, plants may be alternately exposed to nearly fresh water and sea water as the tides ebb and flow. Such species are clearly euryhaline, but only insofar as the lower limit of salinity is concerned. The upper limit of salinity does not exceed that of the ambient sea water. Obviously, tolerance of these species to supersalinity cannot be inferred from their euryhaline nature.

The littoral zone is an environment in which plants are subjected to the greatest extremes of salinity. On the one hand, algae stranded on the beach or on exposed rocks are subject to partial and often extreme dehydration before the tide floods. Even algae growing in shallow tidal pools are subject to increased salt concentration as evaporation progresses. On the other hand, rain may appreciably dilute the sea water in such pools and may temporarily bathe exposed algae in essentially salt-free water. Biebl (1962) states that algae of the intertidal zone, such as species of *Enteromorpha*, *Fucus*, *Porphyra*, and *Ulva*, can tolerate a salinity range of 0.1 to 3.0 times sea water.

Kanwisher (1957) compared the effects of various dehydrating processes (freezing, drying, immersion in hypersaline water) on the survival and metabolism of various intertidal algae. *Fucus* could lose as much as 90% of its water by drying and recover upon re-immersion. Immersion in water three times the salinity of seawater caused only a one-third reduction in respiration. Cellular chloride concentrations of *Fucus* and other intertidal algae were proportional to the chloride concentrations of the hypersaline bathing media. Although obviously resistant to supersalinity for hours and in some cases days, and capable of full recovery upon return to normal sea water, the capacity of intertidal algae for growth when they are *maintained* under supersaline conditions is not known. Salinities that cause marked growth depressions of crop plants have little or no depressive effect on photosynthetic or respiratory capacity (Nieman 1962). Photosynthate may even accumulate to greater than "normal" levels in salt-stunted plants (Bernstein and Hayward 1958). Maintenance of these metabolic functions and metabolite levels does not assure a sustained capacity for growth.

Sublittoral algae, mainly red algae, are intolerant to hypertonic solutions and thus cannot survive if osmolarity of the medium is increased by more than about 50%. Biebl (1962) gives the tolerable-salinity range for these algae as 0.5 to 1.5 times seawater. Again, these figures refer to the range for survival and not the range for growth. The latter is probably narrower.

Planktonic species have fortunately been studied under controlled conditions. Growth of four species in the laboratory at the indicated salinity levels, as reported by Braarud (1951), is shown in Figure 2. *Syracosphaera*, a coccolithophorid whose known distribution includes only brackish or sea-water basins, is nevertheless represented as a euryhaline species with characteristics of algae having oceanic distribution. Its growth curve does not show an appreciable diminution up to 45 ppt. The dinoflagellates, *Amphidinium* and *Exuviaella*, exhibited pronounced reductions in cell division rate at salinities greater than 40 ppt. *Exuviaella baltica*, with a range from the Baltic Sea into the open ocean, is described as euryhaline but neritic. It is significant that these species, tolerant to oceanic salinities, exhibited such a marked decline in growth rate when oceanic salinity was exceeded by only a small degree. The cryptomonad, a species found in coastal waters of low salinity, exhibited the expected greater sensitivity to salinity.

Nordli (1957) performed similar experiments with several species of *Ceratia* (Fig. 3). These dinoflagellates are neritic and not oceanic, but some of these species do occur in the eastern Mediterranean and the Gulf of Oman where salinity is 39 ppt. These more tolerant *Ceratia* also exhibit a progressive decrease in growth with increasing salinity, much as that shown by facultative land halophytes (Fig. 1).

There are, of course, algae that are extremely salt tolerant. These occur in salterns and in brine lakes of western North America, some of which are saturated with salt. *Dunaliella* may even color the salt crusts green in such lakes (Smith 1933). In controlled experiments, *D. salina* grew over a range of 20 to 220 atm (up to ten times the osmolarity of sea water), and exhibited more than two-thirds of its normal photosynthetic and respiratory rates when growth was reduced by 60% in the presence of 1.25 M NaCl (Marré, Servettaz and Albergoni 1958). Such extreme halophytes, comparable to the obligate land halophytes, are not characteristic of the normal marine environment.

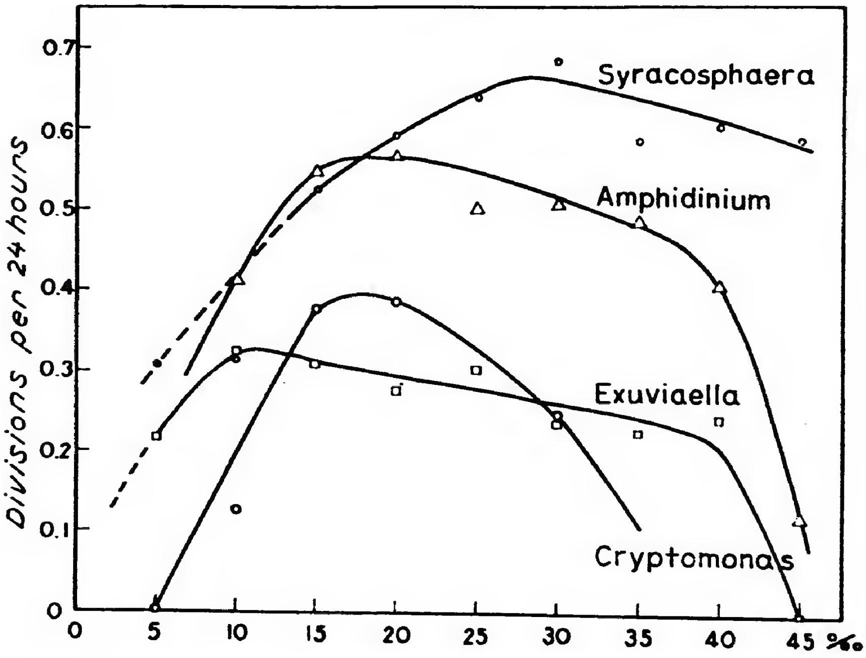

FIG. 2. Effect of salinity on cell division rate of four species of marine phytoplankton. From Braarud (1951).

CONCLUSIONS

Some marine algae are rather stenohaline, particularly the red algae of the sublittoral zone. These cannot even survive exposure to hypertonic media and apparently lack the capacity for osmotic adjustment found in the brown and green algae of the littoral zone. Although the latter can adjust to marked increases in salinity up to at least 3 times seawater, it is not known whether they can grow and reproduce if maintained continually at elevated salinities. Phytoplankton of neritic and oceanic distribution can tolerate somewhat elevated salinity, but their growth rate declines markedly as salinity is increased above 40 ppt. Only the algae of salterns and salt lakes appear to possess a capacity for continued growth and reproduction when salinities reach or exceed several times the concentration of seawater. Customary descriptions of marine algae as euryhaline have considered primarily their tolerance to lower salinity rather than the tolerance to elevated salinity, and the growth of some euryhaline dinoflagellates has been shown to be quite sensitive to a salinity of 1.2 times seawater. Sublittoral red algae and phytoplankton would, therefore, appear to be sensitive to any significant increase of salinity in areas affected by off-shore disposal of brine wastes.

ACKNOWLEDGMENT

The author thanks Munksgaard, publisher of *Oikos* and *Physiologia Plantarum* for permission to reproduce the figures from the papers by Nordli and Braarud.

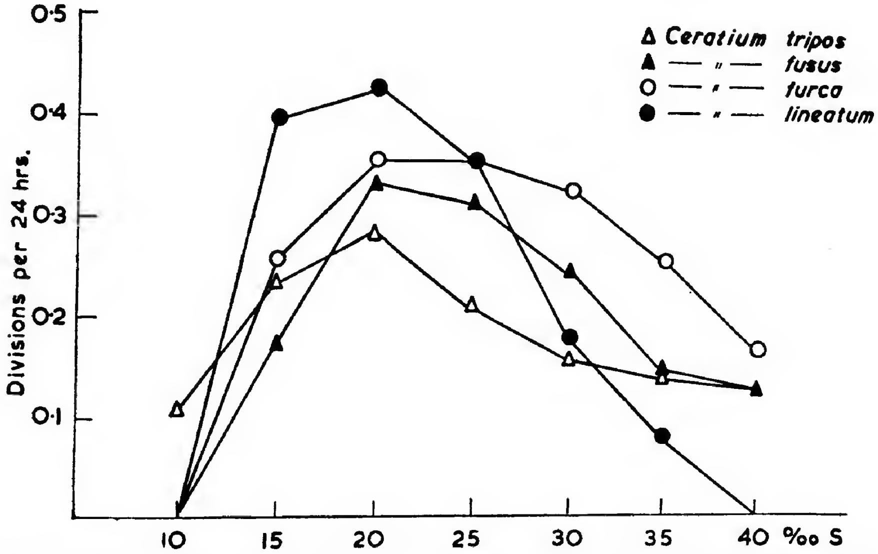

FIG. 3. Effect of salinity on cell division rate of four species of *Ceratia*. From Nordli (1957).

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EFFECT OF HIGHER THAN NORMAL SALINITIES ON SALT MARSHES[1]

H. Dickson Hoese[2]

ABSTRACT

Salt marshes exist in a variety of salinity conditions including (1) where salinity seldom falls below 30 ppt, (2) where waters may become fresh at times and (3) where salinity may exceed 40 ppt. Most, however, exist in waters between about 10 to 30 ppt. Increasing salinity may move the salt-brackish-fresh water boundaries landward as has occurred in Louisiana.

Increase in salinity allows many high salinity organisms to invade landward which may cause immedaite harm to fisheries. Effects of rising salinities are known neither for all salt marsh animals nor for all salt marshes, and may differ. Also as salinity rises there are changes in other important factors that vary among salt marshes. There is little proof that primary productivity will decrease with rising salinity, but it may follow different channels. The United States Atlantic and Gulf salt marsh system based on *Spartina alterniflora* would probably be destroyed as salinity approaches 50 ppt. Effects of lower salinities are much less certain and require intensive study.

INTRODUCTION

Salt marshes are necessarily best developed in areas close to the ocean, where they have a source of sediment and a supply of salt brought in with changes in sea level. The sediment building the marsh is often supplied by rivers which reduce the salinity, therefore permitting landward development of brackish and fresh-water marsh.

Although most botanical studies of marshes have concerned shorelines, with their richer flora, it is well documented that American Atlantic salt marsh is predominately composed of a single species of grass, *Spartina alterniflora.* All other marsh plants are either submergent and seldom exposed (such as *Zostera, Ruppia,* etc.), essentially salt tolerant terrestrial types adjacent to land which are seldom submerged (such as *Distichlis* and *Salicornia*) or brackish types. This distinction is somewhat arbitrary, but serves to eliminate the many forms which cannot survive in open, frequently flooded salt marsh. In the Gulf of Mexico where tides are small, several marsh communities may be closely adjacent. Much *Spartina alterniflora* marsh, for example, has areas where other genera occur on slightly higher and/or fresher ground.

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Most salt marsh has little other macroscopic flora; therefore determining the effect of rising salinities on organisms in a salt marsh requires not only the determination of direct effects on the animals and plants themselves, but also the more difficult determination of indirect effects through influences on *Spartina.* For example, in Georgia, Teal (1962) estimates 6580 k cal^{-2} yr^{-1} net production from *Spartina,* with an additional 1620 k cal m^{-2} yr^{-1} from microscopic marsh algae (Pomeroy 1959). Plankton and macroscopic algae or spermatophyte production is probably prevented by high turbidity (Ragotzkie 1959). This, however, denied by Schelske and Odum (1961) who think some plankton production occurs. Other salt marshes have less turbid water and a well developed submerged flora, which along with phytoplankton should contribute a large amount of primary productivity. The effect of rising salinities either from lack of river flow or from introductions of effluents, immediately presents a number of complexities, most of which are not understood.

DISTRIBUTION OF AMERICAN ATLANTIC SALT MARSHES

An old estimate (Griffiths 1928) stated that over 5 million acres of salt marsh existed in the South Atlantic and Gulf of Mexico states, the main area covered by this report. While much of this has been destroyed by filling and diking, the habitat is a large and important one. Only Chapman (1960) has listed a large number of American marshes, but he omitted many large ones.

Between Texas and Maine, salt marshes are present to some degree in nearly every coastal state (Fig. 1). In South Texas there is no salt marsh in the hypersaline Laguna Madre although some exists near the mouth of the Rio Grande. Small "islands" of marsh called tidal deltas exist in bays to the south and west of inlets. Four of these inside Aransas Pass, Passe Cavallo, San Luis Pass, and Bolivar Roads are well developed, but the latter has been almost completely filled. A small amount of marsh also exists along some shores in the bays between Corpus Christi and Galveston. Little or no salt marsh exists from Galveston Bay to Vermillion Bay, Louisiana. There is scattered marsh from there to about 91° W where a large salt marsh complex extends to Mississippi Sound, interrupted only by the Mississippi River. An important marsh study was done there by Penfound and Hathaway (1938), and much information on Louisiana marshes may be found in O'Neil (1949) and Mackin and Hopkins (1962).

There is very little *Spartina alterniflora* marsh in Mississippi and Alabama, but there is much *Juncus* marsh along northern Mississippi Sound. This marsh, composed primarily of *Juncus roemerianus*, is common in the Gulf of Mexico, usually existing landward of *Spartina*.

The northwest coast of Florida from Pensacola to Apalachee Bay consists mainly of open lagoons and estuaries with little marsh. From Apalachee Bay south to Tampa is a poorly described area, but some marsh of unknown extent exists in the area. A major study (Kurz and Wagner 1957) on salt marshes described several from Apalachee Bay. According to Curl (1959), the area between the Ochlockonee River and Cedar Key is composed of *Juncus* marsh. A study of the fishes of two marshes in the area was done by Kilby (1955).

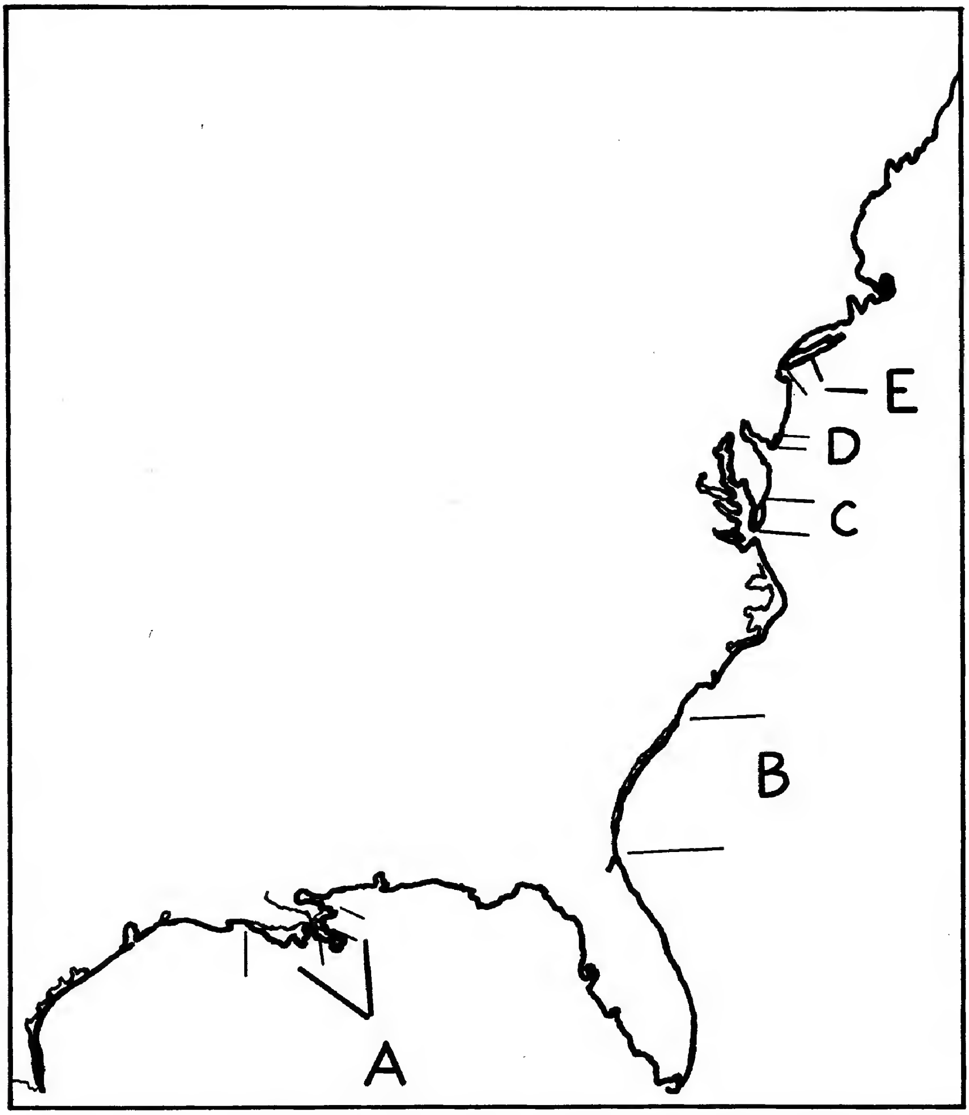

FIG. 1. Major areas where salt marshes compose a significant fraction of available estuarine water. A = Louisiana; B = Georgia and South Carolina; C = Virginia Seaside; D = lower New Jersey and Cape May peninsula; E = western Long Island.

On the Atlantic Coast from Cape Canaveral to Jacksonville, Florida, there is little marsh, but beginning at about the St. John's River and extending to near Georgetown, South Carolina, is the largest undisturbed salt marsh remaining on the coast. The marsh fills most of the area between the islands and mainland, except at river mouths and the sounds draining the marsh.

There are narrow but well-developed salt marshes at Murrell's Inlet, South Carolina, and extending from Cherry Grove Beach, South Carolina, to Shallote Inlet, North Carolina. A similarly narrow marsh may exist at the mouth and north of the Wilmington River, but I am not familiar with the area. An area similar to the Texas tidal delta exists inside Bogue Inlet, N.C. and there are several marshes around Morehead City (Reed 1947; Davis and Gray 1966). Most of the North Carolina Sounds have some peripheral marsh, but the next large marsh begins on the outer Virginia coast (Seaside) from Smith Island to Chincoteague. Also, there are numerous small marshes developed in Chesapeake and Delaware Bays.

The next system, very similar to that on the Virginia Seaside extends from Cape May to just north of Atlantic City, New Jersey. Smaller marshes exist in the remainder of New Jersey. The western part of Long Island is another system somewhat comparable to those of the New Jersey and Virginia peninsulas. The marshes of this area as well as those to the north have been studied by several authors (Chapman 1960). One major early study, especially of botanical interest, was done on the northern shore of Long Island (Johnson and York 1915).

SALINITY

The physiography of these salt marshes differs considerably, as does the salinity. The best developed marshes of Louisiana and Georgia have the most widely fluctuating salinities. Most Gulf tides are diurnal and less than 2 feet, causing only small daily variations of few ppt except near the inlets (Mackin and Hopkins 1961), whereas in the Southeastern United States system, twice daily changes of over 10 ppt are common (Galtsoff and Luce 1930). In both areas, fresh-water covers salt marsh for short periods, and only those farthest from rivers maintain salinities much above 20 ppt.

A summary of some salinity information is shown in Table 1. These data include both marsh water and soil salinities, and while the latter are a close reflection of water salinities, they are somewhat more stable but usually higher (Penfound and Hathaway 1938). In Figure 2 three well developed marsh systems with seasonal data are compared along with a daily change in one system. One is an extreme, a small marsh on a tidal delta at Harbor Island, Texas. Here salinities have never been known to go below about 20 ppt and in the year of record never were below 32 ppt, during the dry summer commonly exceeding 40 ppt. Small patches of *Spartina* in South Texas are occasionally exposed to higher salinities, but the Laguna Madre with salinities usually above 40 ppt (Simmons 1957) has no *Spartina*. However, the Laguna Madre is far removed from both the ocean and a sediment source.

Another type of salinity regime exists in the salt marshes of the Vriginia Seaside (Fig. 2). Here, there is little land drainage and salinity is almost always be-

TABLE 1

Summary of some salinity data from American Atlantic salt marshes. Data given in water salinity (ppt)

Authority	Location	Salinity	Comments
Hewatt (1951)	Barataria Bay, La. area	0–30+	Extensive data in marshes and adjacent bays
VIMS	Seaside of Virginia	17–36	Most data unpublished but extensive
Copeland and Hoese (1966)	Harbor Is., Texas	32–24	Few readings over 1 yr period
Galtsoff and Luce (1930)	Georgia Coast Doboy Is., Ga.	0–24	Scattered readings in Feb., Mar.
Drake (1891)	Georgia coast	0–29	Effect of tide at one station
Green and Lunz (1950)	Wadmalaw Is., S. Ca.		Scattered specific gravity readings
Johnson and York (1915)	Cold Spring Harbor, L. I.	8.6–28.2	Extensive daily readings
Data given in soil salinity (%):		30	Stated as normal high tide salinity
Allan (1950)	Gulf coast	3–4.6	Summary figure
Penfound and Hathaway (1938)	Mississippi R. delta	0.55–4.97	
Taylor (1939)	Long Island	0.75–3.25	
Harshberger (1909)	New Jersey	0.25–4.25	
Kurz and Wagner (1957)	Florida and South Carolina	0.10–4.64	

tween 25 and 35 ppt. However, the inner marsh channels may have salinities which drop after heavy rains to one half.

In the salt marshes of Georgia and South Carolina, almost all salinity types may be found. Average tides are over 2 m and occasional spring tides exceed 3 m. Near rivers, the salinity changes tremendously with each tidal cycle (Galtsoff and Luce 1930; Fig. 2). At intermediate points between major rivers, the salinity

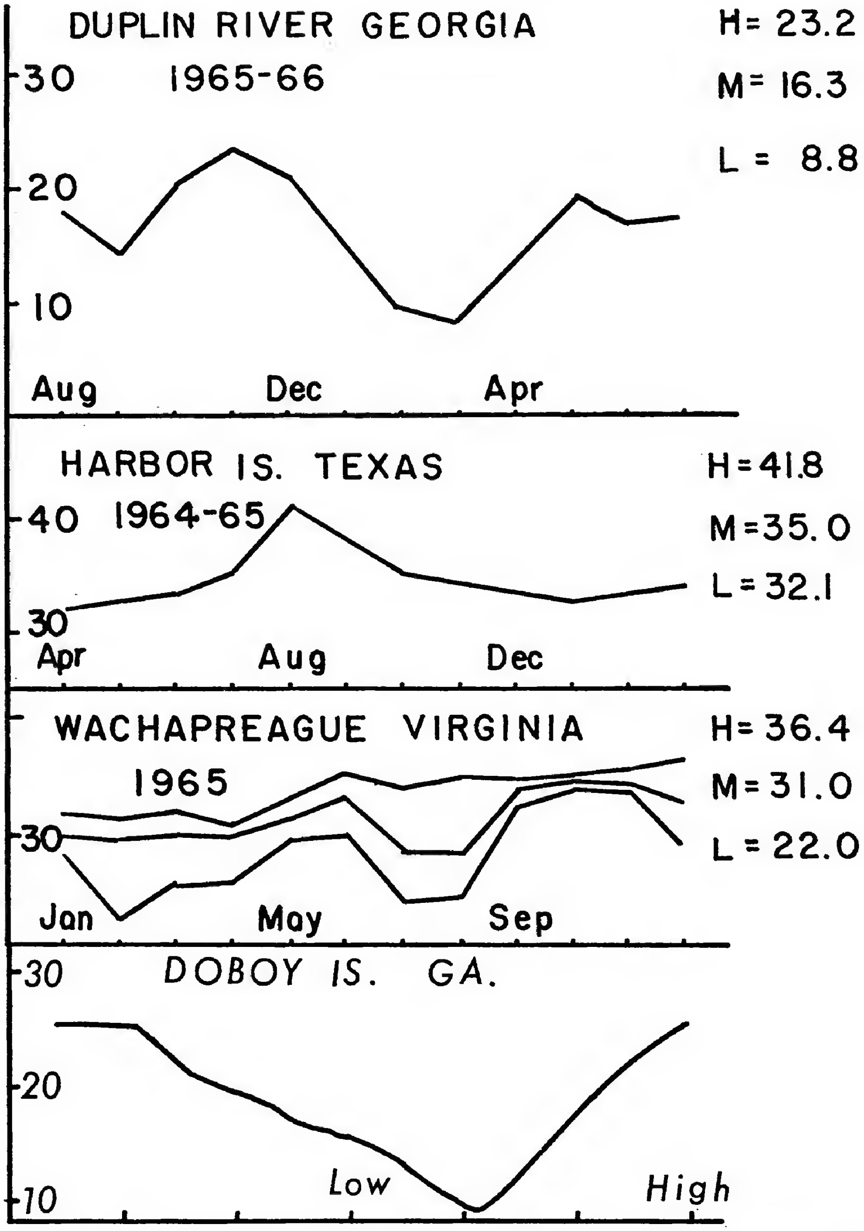

FIG. 2. Seasonal salinities (ppt) in three salt marsh areas and tidal effect in one marsh (from Galtsoff and Luce 1930).

is presumably less variable and some data we have for 1966 indicate it is usually over 20 ppt. Data from a location which is not the highest salinity marsh in the southeast are shown in Figure 2. In such tidal situations the *Spartina*, which grows above average sea level, is covered only at high tide. Therefore, lower salinities existing in the channels may never actually cover the marshes but be pushed upstream while higher salinity ocean water floods the grass. This means that the organisms in the tidal channels can be subjected to lower salinities than those in the marsh proper.

EFFECTS OF RISING SALINITIES

There is probably more known about the distribution of marine organisms in open estuaries than in salt marshes. Many of the organisms are similar and presumably what has happened in open bays will also occur to some degree in salt marshes.

In the later 1940's and early 1950's a severe drought affected the midwest, reaching an extreme in 1956. On the Texas Coast, many rivers finally stopped flowing and others dropped to a fraction of their former flow. Although data are incomplete, bay salinities rose until in 1954–56 salinities seldom fell below 30 ppt in the bays on the central coast. During this period, there was widespread invasion of the bays by stenohaline marine organisms and some of the former estaurine bay fauna disappeared (Puffer and Emerson 1953; Parker 1955; Hoese 1960). The fisheries organisms most damaged were the blue crab and white shrimp, which depend on low salinity areas for development of young, and the oyster, which requires fresh water to destroy its enemies. At the same time, hard clams (*Mercenaria*) invaded the bays providing other potential but unused fisheries.

Whether the same events would happen in salt marshes when salinities rose is hard to say. In Louisiana salt marshes and bays rising salinities have definitely damaged the oyster fishery (Mackin and Hopkins 1961). Oysters are, however, successful in the intertidal zone of marshes where high salinity predators cannot reach them. Oyster drills (*Urosalpinx* and *Thais*) could be expected to become more widespread as salinities rise and probably raise the lower tidal level where oysters would grow, thereby reducing the available area. This could be partly offset by invasion of hard clams, which now provide fisheries in many high salinity salt marshes.

The only work I find predicting the effect of rising salinities on marshes is by Rounsefell (1964), who estimated the effect of a ship channel through the Louisiana marsh. He concluded that a rise in salinity of a few ppt caused by a deep water ship channel would harm blue crabs, oysters, and perhaps white shrimp. He also concluded that most of the fish fauna will be unharmed because they are euryhaline, but some of the species are no more euryhaline than are crabs, oysters, or shrimp.

Copeland (1966) was concerned with the effects of reduced river flow and subsequent increased salinity on estuaries in general and their fauna. From a review of the literature he presents evidence that rising salinities: (1) damage oysters by invasion of parasites and predators, (2) cause decrease in the total shrimp catch, (3) kills blue crabs because of necessity of freshwater for development of young stages, (4) may affect or change primary productivity and (5) affects many fishes whose juveniles enter low salinity water. He presents the hypothesis that in Texas bays there is an optimum river flow (somewhat above the average for most bays) that is necessary to maintain maximum commercial production of seafoods (mainly shrimp and oysters). A similar relation between river discharge and commercial production has been shown by Chapman (1966).

There are two peculiar factors that seem to be related to high salinity salt marshes. In such marshes in Virginia, Texas and Georgia the oyster fungus, *Dermocystidium marinum,* is rare to absent. It is unknown in Virginia (Andrews and Hewatt 1957; also unpublished data) and in Texas marshes (Hoese 1964), but in slightly lower salinity Georgia marshes the parasite is common to absent, decreasing with rising salinity (Table 2). In lower salinity marshes in Georgia, Louisiana, Texas and probably elsewhere the disease is common, contrary to its normal distribution in open estuaries where it increases with rising salinity (Mackin 1961).

Another phenomenon that may be related to marshes is giantism in oyster drills. Large numbers of the genera *Urosalpinx* and *Thais* are known only from salt marshes in the Southeast (unpublished data) and the Virginia Seaside (Henderson and Bartsch 1914), but it is not certain if giantism is caused by the marsh. If giantism is induced by something from the marsh, it is further evidence of the distinctiveness of marshes.

The abundance of juvenile forms in low salinity areas and their lesser abundance in higher salinities is well known. In salt marshes both near to and distant from inlets I have often observed and captured large numbers of juvenile fishes and invertebrates in moderate to high salinities suggesting that these marshes may offer some of the same necessary factors as open areas of fresher water estuaries. Unfortunately, there are few data on the subject. A recently completed study on salt marsh fishes by Clarence Richards of the Virginia Institute of Marine Science will help greatly in predicting the extent of such invasions.

Another possible effect, true perhaps of estuaries in general, is the danger of removal of orientation compounds that marine animals use for migrating inland. Use of orientation compounds has been shown for only one marine species

TABLE 2

Distribution of the parasitic oyster fungus, *Dermocystidium marinum,* in Georgia salt marshes, January through June, 1966, grouped according to the presence of oyster drills, *Urosalpinx cinerea,* and by salinity.

Area	Number of oysters examined	Infected		Salinity range (ppt)
		Number	Percent	
Oyster drill areas remote from river mouths (over 15 mi)	180	0	0	20–33
Intermediate areas (no drills)	360	19	5.3	13–28
Low salinity areas near river mouths (under 5 mi)	700	155	22.1	0–20

(Creutzberg 1961), but if it proves to be a general phenomenon, lack of river flow or masking of these compounds by effluents may interrupt migrations of many species. This would be especially important to the larvae of commercial species entering Atlantic and Gulf estuaries.

Another factor, not produced directly by rising salinities but related, is lowered estuary water levels, especially close to river mouths. Removal of fresh-water inflow while permitting landward intrusion of salt water lowers bay water levels so that many shallows normally productive of juvenile invertebrates and fishes become dry land. This problem has not been directly investigated, but one study shows a relation between abundance of animals in shallows and sea level (Hoese and Jones 1963). This may not be as critical on the Atlantic Coast where larger tides obscure small water level changes, but on the Gulf differences in levels of a few inches can be significant in terms of land area covered. This becomes less important near the ocean where most salt marshes occur.

Another important effect of rising salinities may be the intrusion of sharks along with other stenohaline animals. The high salinity salt marsh area of the outer Virginia coast contains an impressive abundance of sharks (Hoese 1962). Lower salinity Georgia marshes have few sharks, but seem to have large populations of longnose gar, *Lepisosteus osseus*. Of course, removal of the salinity barrier would also allow invasion of innumerable species of both plants and animals.

Finally, rising salinities could replace brackish and fresh-water marshes with salt marshes. This might be affected, however, by ion antagonism, which could perhaps be induced by some supersaline effluents. A discussion of the effect of single ions and ion imbalances on salt marsh plants is found in Chapman's (1960) book.

While the causes of nearly all these phenomena are speculative, it is not illogical that salt marshes differ from open estuaries in some respects. Marshes have a large ratio of sediment surface area to volume and this could result in much biochemical activity. For example, ZoBell and Feltham (1942) found that bay mud contains many more bacteria than the water. It follows, therefore, that the large amount of mud surface area in marshes might produce more or even different biochemical activity than the average estuary.

Since the distribution of many marsh organisms is controlled by salinity, increasing marsh salinities may cause changes in numerous factors, which effect complex interactions that we have neither the data nor the imagination to deal with at present.

EFFECTS OF HYPERSALINITY

Based on the known distribution of plants in Texas and salt tolerance tests (reviewed by Chapman 1960; Table 1), it can be assumed that hypersalinities of 40–50 ppt would kill *Spartina alterniflora* and that no other higher plant would take its place. The effect of removal of *Spartina* on sedimentation is uncertain, but many changes could be expected to occur. If the sediment supply were from a river, the marsh mud might be expected to show a net erosion, assuming present marshes are in some sort of equilibrium. This agrees with the discussion by Chapman (1960: p. 36).

According to Spector's (1956) list the salt tolerance of marsh organisms is similar to that of *Spartina* and only the organisms characteristic of a hypersaline lagoon or salt flat would remain. Destruction of *Spartina* would remove a source of food for many organisms, but perhaps a reduction in turbidity from lack of new sediment and no more decomposing plants would permit increases in phytoplankton and benthic algae photosynthesis.

It is uncertain, however, if such hypersaline conditions can be maintained short of extreme conditions. The best developed marshes exist close to a tidal situation, usually near an inlet; therefore, water covering the marshes is often ocean water, and further increases in salinity would be far slower than increases from low and moderate to oceanic levels.

EFFECTS ON PRODUCTIVITY

Studies of productivity in marine waters have progressed little beyond primary productivity and there has been little consideration of the effects of salinity. Copeland (1966) notes that estuaries may be more productive at river mouths, but that floods can dilute nutrients. While this may decrease estuarine production, it can also provide nutrients for less productive offshore areas (Thomas 1966).

It may be that salt marshes are more or less self-contained units which depend little on outside waters. Certainly many marshes, productive of fisheries at least, have negligible river flow, and the studies in Georgia have produced little evidence that river flow is very important, but rather the rapid recycling of nutrients within the system helps maintain high productivity (Schelske and Odum 1961) and floods may dilute these nutrients (Thomas 1966). This idea is consistent with Mansueti's (1962) hypothesis that open estuaries may receive some benefit from unusual amounts of nutrients.

It has been suggested that estuaries are more productive in higher salinities, even hypersalinities (Odum and Wilson 1962), but Rounsefell (1963) has pointed out that this could be damaging to many fisheries. Such suggestions do not consider the possibility that there may be some direct uses of organic carbon by estuarine animals which compensate for turbidity reduced photosynthesis.

In this regard, Stephens (1964) reported that a marine worm, *Clymenella*, was able to take up glycine from its water, but only at salinities higher than 12 ppt. The abundance of amino acids in estuaries is becoming known (Park *et al.* 1963), and conceivably their utilization could be enhanced by increases in salinity. This, again, is an area requiring much study before suitable predictions may be made.

Apparently, *Spartina alterniflora* grows better experimentally under brackish conditions than in oceanic salinities (Taylor 1939). Whether this would result in decreased system productivity in high salinities cannot be determined. The productivity studies in Georgia have all been conducted in the vicinity of the Duplin River where salinities are brackish (Fig. 2).

One final thought, touched on in the introduction, is that rising salinities, when accompanied by reduced turbidity, may allow more phytoplankton and benthic algae photosynthesis.

CONCLUSIONS AND SUMMARY

Throughout this paper speculative license of a reviewer has been widely used, but almost always based on a set of facts fairly well documented. Unfortunately, further study may destroy the validity of these speculations. Their value, therefore, lies in emphasizing two points: (1) the simple rise in salinity of a marsh results in or is accompanied by a complex of changes, some which seem beneficial, some which seem harmful, but none which seem to alter drastically the marsh until hypersalinities are reached; and (2) we are probably on the verge of discovering a number of interactions in marshes which are in some way related to salinity and could change as salinities change.

One good test of a work, after all is said, is to ask the question: what could be recommended if some agency wanted to raise marsh salinities? If salinities would become higher than oceanic and the character of the march were threatened, to save the marsh the answer would seem to be a simple "do not do it." But if a moderate salinity marsh would be raised to oceanic salinity levels without salt imbalances what then? *Status quo* may be the best for most marshes, at least until we have enough information to predict better the total effect of salinity changes. It also seems certain that changes will occur in populations of many organisms, some of which may be fished by man and potentially destroyed. Also, the local fauna would be expected to be adapted to a special set of local conditions. Such a possibility makes generalizations for all marshes dangerous, because, despite basic similarities of all salt marshes, there are significant differences in many factors important to life.

This paper will end in a plea, which is already partly evidenced and answered. The pressure of rising salinities will increase, as first Texas and then the northeast have experienced, and while marshes may withstand the change better than most estuarine waters, all interests should make certain of this. After all estuaries are only downstream from the whole nation.

ACKNOWLEDGMENTS

I am greatly indebted to the Virginia Institute of Marine Science, The University of Texas Marine Science Institute and the University of Georgia Marine Institute, who have made it possible for me to work in salt marshes. Paul Chanley and Mike Castagna made salinity data from Virginia available. Salinity data from Georgia marshes were made possible by support from the Sapelo Island Research Foundation and the Georgia Game and Fish Commission. Dr. Henry Hildebrand of the University of Corpus Christi contributed information on the distribution of *Spartina* in South Texas. The help of the Inter-Library Loan Department of the University of Georgia greatly facilitated examination of the literature.

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INTRODUCTION OF COMMERCIALLY IMPORTANT SPECIES INTO INLAND MINERAL WATERS, A REVIEW

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ABSTRACT

The successful introductions of commercially important animals into inland mineral waters of the following four categories were reviewed.

1. *Lagoons.* These have been modified into ponds and used for the culutre of marine animals in Europe and Asia. Milkfish, *Chanos chanos,* cultured most extensively in Asia can survive in ponds with salinities as high as 140 ppt. Mullets, *Mugil* spp., prawns, *Penaeus* spp., and other animals can survive up to 70 ppt in these ponds. In Europe, ells, *Anguilla anguilla,* mullets, *Mugil* spp. and other fishes are cultured in salinities up to 47 ppt.
2. *Relict waters.* To improve the food base for fish, man has introduced additional species of fish and invertebrates into the Caspian Sea (salinity 12 to 13 ppt) and the Aral Sea (10 ppt). The mullets, *Mugil auratus* and *M. saliens,* were introduced and now support an important fishery in the Caspian Sea. *Nereis diversicolor* was introduced as a food for fish in the Caspian Sea. The Baltic herring, *Clupea harengus,* has been introduced into the Aral Sea.
3. *Salterns and inland brines.* Man has successfully introduced fishes into the Salton Sea, California (salinity 33 ppt), and Lake Quarun, Egypt (29 ppt). Nearly the entire fauna of the Salton Sea, in a food chain leading to a sport fish, *Cynoscion xanthulus,* was introduced. Mullets, *Mugil cephalus* and *M. capito,* and the sole, *Solea vulgaris,* were introduced into Lake Quarun and are the objects of a commercial fishery.
4. *Carbonate and sulfate waters.* Whitefish *Coregonus clupeaformis,* were introduced into the Quill Lakes, two saline lakes of Saskatchewan, when the salinities were 11 ppt and 17 ppt. They

supported a commercial fishery for about 10 years until the salinities reached about 19 ppt. Other introductions were made in lakes of various salinities in the region.

The success of the introduction of commercially important species into these waters suggests that further attempts should be made to introduce animals into hypersaline waters. Effluents from desalinization plants could be used for pond culture of fishes and invertebrates. No waters are too saline for the culture of species useful to man; the economically valuable brine shrimp, *Artemia salina*, tolerates saturation.

INTRODUCTION

Man has made few attempts to introduce useful species of animals into hypersaline waters. There are, however, a number of studies that report the successful introduction of commercially important species into inland mineral waters. Characteristically, these inland mineral waters are of varying and increasing salinity. Hedgpeth (1959) gave a classification of inland mineral waters which provides a useful outline. They are:

1. *Hypersaline lagoons with permanent or intermittent connections to the sea.* European and Asiatic pond culture of prawns and fish takes place in man-made or man-regulated lagoons, in which the salinity varies from fresh to near saturation.
2. *Relict waters in which the presence of marine organisms shows a former connection with the sea.* Man has successfully introduced animals into the Caspian Sea (salinity 12 to 13 ppt) and Aral Sea (salinity 10 ppt).
3. *Salterns and inland brines containing principally NaCl.* Almost the entire fauna of the Salton Sea (salinity 33 ppt) was introduced by man, as was the major part of the commercially important fauna of Lake Quarun (salinity 29.4 ppt).
4. *Carbonate and sulfate waters.* Commercial fishes have been introduced into some of the saline lakes of Saskatchewan, whose salinities vary from fresh to near saturation.

SALT WATER LAGOONS AND PONDS

Saline ponds are potentially useful for the increased production of food. Culture of animals, and of plants as their food, in these ponds is a well-developed art that dates back at least 700 years in southeast Asia, and whose origin in Europe is lost in antiquity (Schuster 1952; Frey 1947; Herre 1953b; 1953c; D'Ancona 1954; Chimits 1955; Gopinath 1955; Blanco and Acosta 1958; Kesteven and Job 1958; Vatova 1962).

Ponds have been constructed in the regions of river deltas, in some areas by blocking off a natural bay or lagoon and in others by excavation and use of the excavated soil to embank the pond (Schuster 1952). In these situations the water supply is variable and, as a result, the salinities of ponds vary according to location. The salinity of a particular pond may also vary seasonally by a considerable amount. Djaingsastro (1955) reported salinities in excess of 140 ppt in ponds in Java, and Vatova (1962) reported salinities up to 47 ppt in the "valli salse da pesca" of the Adriatic.

Ponds cover large areas and provide sizable economic returns. In Java (Indonesia) the ponds covered 316,000 acres (128,000 hectares) and produced a crop valued at more than $100,000,000 per year (Ahjar and Tasripin 1955). About 150,000 acres (60,000 hectares) of ponds in the Philippines (Frey 1947) produced 70,000,000 pounds (32,000 kilograms) of fish per year, which were worth $15,000,000 in 1953 (Herre 1953a). Frey (1947) estimated that there were potentially 1,000,000 acres (400,000 hectares) of usable swamps that could be converted to ponds in the Philippines. The "valli" of the Adriatic covered 49,662 acres (20,098 hectares) and the annual value of their production in 1952 was estimated at $1,600,000 (D'Ancona 1954).

Fish culture in salt-water ponds is also practiced in Hawaii, where it apparently is a holdover from aboriginal times (Cobb 1905), and in India, Hong Kong, Singapore, Viet Nam and Thailand (D'Ancona 1954). Salt-water pond culture has been proposed for Australia (Thomson 1955) and other parts of the world (Shelbourne 1964). Tampi (1958) estimated that India had potentially 1.3 million acres (500,000 hectares) of fish farms. In the United States, however, salt-water pond culture of edible animals has been carried out only on an experimental basis, in a few ponds (Johnson 1954; Lunz 1955; 1956; 1958; Shaw 1965). In other than these experimental ponds, fish culture in saline ponds in the United States has not progressed beyond the primitive stage of harvesting species when they enter natural bays and lagoons. This, in spite of the fact that Lunz (1958) presented an economic comparison demonstrating a highly favorable return on an investment in pond construction and culture of shrimp compared to the return on the same investment in a shrimp trawler. A $40,000 shrimp trawler might yield a high of 50,000 pounds (23,000 kilograms) of shrimp per year, whereas, the same capital would purchase 80 acres (32 hectares) of shrimp ponds with much lower operating costs than would be required by the vessel and might yield 40,000 to 80,000 pounds (18,000 to 36,000 kilograms) per year (estimate based on yields in India).

Shaw (1965) stated that commercial pond culture of oysters in the U.S. is 100 years behind that of Europe. Salt-water pond culture of fishes and shrimp in the U.S. is at least 700 years behind that of Asia. As we all know, the trend in the U.S. is to dredge or fill estuarine lands, or to divert the inflow water. These changes lead to reduced production of shrimp and fish. Saloman (1965) showed that dredge-and-fill operations have reduced bait shrimp habitat in Tampa Bay, Florida, by 80 to 90% since 1920; in 1954 to 1962, the catch declined from 4.5 to 0.3 million shrimp. Many similar examples could be cited.

Chapman (1960) proposed that the utility of such areas is to be realized by damming off and draining them so they can be put into agricultural production. As a counter proposal, I suggest that they be dammed, flooded to a depth of 3 or 4 feet and stocked with appropriate species of fish or shrimp.

Culture of milkfish, Chanos chanos. Culture of *Chanos* is on a larger scale than that of any other salt-water species. It is the principal species of culture in the salt-water ponds of Java (Schuster 1952) and of the Philippines (Frey 1947; Herre 1953a; 1953b). Important pond culture for *Chanos* is also carried out in the Celebes, Sumbawa, Formosa, North Sumatra and Hawaii.

Chanos is a marine fish that occurs in the Pacific Ocean from the Red Sea and east coast of Africa to the coast of Central America and the Gulf of California, and from southern Japan to New Zealand. Herre (1953b, p. 457) stated "This means that some day our descendants may see bañgos (milkfish) cultivated over much of this vast area, with great benefit to the people inhabiting a large part of the Indo-Pacific island world, as well as the lowlands of the south and southeast of Asia."

Djaingsastro (1955) observed the lethal salinities for certain species and found the lethal salinity for *Chanos* to be 140 ppt, when the salinity was gradually increased.

Curiously, the milkfish is not important in the commercial catch in the sea, but achieves its importance almost entirely through pond culture (Schuster 1952; Herre 1953a; 1953b). *Chanos*, an herbivorous fish, feeds low on the food chain, and achieves large production. Details on the culture methods practiced can be found in Schuster (1952) or Herre (1953b; 1953c). Since the fish does not spawn in captivity, fry are collected in the sea and transported to the ponds. Because milkfish must be obtained from the open sea, pond culture depends on introduction of larvae and fry. The capture of young and their transportation, sometimes over great distances, is the basis of an industry in itself.

Culture of tilapia, Tilapia mossambica Peters. Recently introduced into pond culture is *Tilapia mossambica* Peters. Chimits (1955) provided an excellent review of the culture of tilapia and its history.

At one time it was thought that tilapia would replace the milkfish as the principal fish in brackish-water pond culture. The market price is lower for tilapia, however, than for the milkfish and prawns. In addition, tilapia is subject to stunting if not cropped heavily or if the waters are not heavily fertilized. Thus, the harvest may consist of fish not of prime marketable size; tilapia is now regarded as a pest in some parts of the Philippines (Rabanal and Hosillos 1958).

Tilapia culture has recently declined somewhat after an initial rapid development (Chimits 1957). The discovery that all-male offspring results from a cross of subspecies of *T. mossambica* may make possible the control of the stocking rate without reproduction in ponds and lead to a revival of interest in tilapia culture (Hickling 1963).

Tilapia mossambica tolerates salinities in the range of fresh to 40 ppt, but does not reproduce above 30 ppt (Chimits 1955). Stunting, as a consequence of overpopulation, can, therefore, be prevented by keeping salinities in ponds greater than 30 ppt. The species was first introduced from Africa into Java in 1939. During the war, the Japanese introduced it into most of the islands of Java (Indonesia), Malaya and Formosa for food production.

Chimits (1955; 1957) listed 38 countries where tilapia is used in fish culture and 10 countries in which it has become established in the wild. More recently, the species has become established in the United States. St. Amant (1965) reported it in California and Springer and Finucane (1963) reported a related species, T. *heudeloti*, in Florida.

Culture of mullet, Mugil spp. In most of the countries where the milkfish are cultured, mullets are also raised (Herre 1953c; Blanco and Acosta 1958; Schuster

1952). Most operators attempt to maintain pure cultures of milkfish, but in some ponds juvenile mullet are accidentally carried into the pond during filling.

Five species of mullet (Table 1) are cultured in the salt-water ponds of the Adriatic in salinities as high as 47 ppt (D'Ancona 1954; Vatova 1962). In this region, the mullet fry are sought in the ocean and stocked in the ponds. Mullet are also raised in brackish-water ponds in other parts of the world; *e.g.*, India (Tampi 1958), Hong Kong (Herre 1953c) and Japan (Blanco and Acosta 1958). They are harvested from ponds in 6 to 8 months, as are the milkfish.

The upper limit of salinity tolerated by *Mugil* is not known but is probably near 100 ppt (Breuer 1957). The highest salinity recorded by Simmons (1957) in waters of the Texas Laguna Madre, where *M. cephalus* are found, was 80 ppt. Zenkevich (1963) reported *M. cephalus* in the Sivash, a lagoon in the Sea of Azov, in a salinity of 60 ppt. Djaingsastro (1955) found that a mullet, *Mugil* sp., did not survive at salinities above 70 ppt in the salt-water ponds of Java.

Miscellaneous species cultured in salt-water ponds. Some 15 species of shrimps and prawns are reared in salt-water ponds in Asia and the far east (Kesteven and Job 1958). In India, prawn culture is carried out in alternation with cultivation of rice in over 10,000 acres (4,000 hectares) which produce about 9,600,000 pounds (4,400,000 kilograms) of prawns per year (Gopinath 1955). Salinities are normally less than 32 ppt. Djaingsastro (1955) reported that lethal salinity for prawns in Indonesian ponds was near 70 ppt. Complete instructions for the culture of a shrimp, *Penaeus japonicus,* including its spawning and rearing of larvae in aquaria until they are large enough for ponds, can be found in Fujinaga (1963). This species reaches commercial size in 1 year.

The puffer, *Fugu rubripes* (Temminck et Schlegel), is cultured in seawater ponds in Japan (Takai *et al.* 1959). Salinities of the ponds are variable and some have little or no tidal exchange. Fifteen other species of fish occur in these ponds. *Mugil cephalus* is very common.

In addition to the five species of mullet mentioned above, the salt-water ponds of the Adriatic are used for eels, *Anguilla anguilla* L., gilthead, *Sparus auratus* (L.), and sea bass, *Morone labrax* Cuv. (Table 1). All are introduced as elvers or fry. A number of other species occur incidentally, the most important of which is a flounder, *Pleuronectes flesus* (D'Ancona 1954).

TABLE 1

Species of fish reared in salinities to 47 ppt in the valli salse da Pesca[1]

Common name	Scientific name
Eels	*Anguilla anguilla* L.
Gilthead	*Chrysophrys aurata* L.
Bass	*Labrax lupus* Cuv.
Mullet	*Mugil auratus* Rissp
Mullet	*M. capito* Cuv.
Mullet	*M. cephalus* Cuv.
Mullet	*M. chelo* Cuv.
Mullet	*M. saliens* Risso

[1] From Vatova (1962).

Culture of oysters in salt-water ponds has been carried out extensively in Europe (Dean 1892; 1893). Recently, his idea that oyster culture should be tried in the U. S. has been revived (Lunz 1956; Loosanoff 1956; Carriker 1959; Shaw 1965). In spite of some promising experimental work, commercial application of the knowledge has not been attempted.

Harvest of brine shrimp, Artemia salina. When salinities become excessive for fish, or in isolated or temporary salt ponds in which there are no predatory fish, the brine shrimp is almost certain to be found. It spreads by way of its amazingly resistant eggs or cysts, which are carried by the wind, by birds and other animals; or they may simply remain dormant on the dry earth for years. The eggs can survive a temperature of —190 C for 24 hours and extreme lack of moisture and oxygen as shown by their survival for 6 months at high vacuum, 10^{-6} mm Hg (Whitaker 1940). The adults live in salinities up to saturation and can reproduce at that concentration (*see* Littlepage and McGinley 1965).

An important industry for brine shrimp exists in the United States, primarily to supply food for aquarium fishes (Dempster 1953) and, more importantly perhaps, food for aquatic laboratory animals of all sorts. At present three commercial operators are licensed to harvest brine shrimp eggs from the shores of Great Salt Lake (D. C. Hales, personal communication). In 1964, they harvested 173,000 pounds (78,000 kilograms) of brine shrimp eggs (Donald Andriano, Utah Department of Fish and Game).

Brine shrimp eggs are not difficult or expensive to harvest because they float to the surface and are washed ashore in windrows. Hales and Bangerter (1957) reported finding 1,000 pounds (450 kilograms) of eggs on the shore of Great Salt Lake in an area 10 by 50 feet (3 by 15 m). They estimated a cost of 8¢ per pound for collecting the eggs.

Adults and eggs are also harvested incidental to salt production in evaporation ponds in San Francisco Bay and San Diego Bay. Highest prices are obtained for the eggs which are vacuum packed and sold by the gallon. There are about 4 pounds per gallon (0.5 kilograms per liter) and they are valued between $3.00 per pound for Great Salt Lake eggs and $8.00 per pound for San Francisco Bay eggs which supposedly yield larger, more nutritive nauplii.

I estimate that the three Great Salt Lake operators grossed over half a million dollars in this business in 1 year. Schmitt (1965) estimated the value of the brine shrimp operation at $350,000 in San Francisco Bay, $25,000 in San Diego Bay and $30,000 in Mono Lake, California. The value of the harvest of brine shrimp adults and eggs from two saline lakes in Saskatchewan, Canada, varied between $150,000 and $450,000 a year from 1963 to 1965 (value estimated from harvest figures supplied by G. E. Couldwell, Department of Natural Resources, Saskatchewan, personal communication).

RELICT WATERS: CASPIAN SEA AND ARAL SEA

CASPIAN SEA

The Caspian Sea is partly of fresh-water and partly of marine origin, as reflected in its fauna. Being 92 feet (28 m) below sea level, it has no outlet and its salinity is destined to increase gradually as a result of evaporation. Evaporation carries off a volume of water equal to the inflow. The net effect of the inflow is to add the minerals it carries to the minerals in the Caspian Sea and thereby increase the salinity. It is the world's largest landlocked body of water; the area is 275,000 square miles (712,000 square kilometers). Its salinity is 12 to 13 ppt. Evaporation from the surface is 2.8 feet (86.6 cm) per year. Shallow bays on its eastern shore have salinities that exceed 200 ppt. The high rates of evaporation from some of these areas, which must be balanced by inflow from the Caspian Sea, may be a mechanism which retards the increase in salinity of the Caspian Sea proper (Twenhofel 1932, as quoted in Emery and Stevenson 1957).

The 78 species of fish in the Caspian Sea accounted for 65.4% of the 1954 Russian fisheries yield. (This percentage must be much less today because of the recent increase in Russian fishing in the world's oceans.) The 78 species represent 17 families; of fresh-water, Mediterranean and Arctic origins.

Between 1900 and 1951 the introduction of 14 species was attempted, but only 8 were established. Four Mediterranean species of fish, of three families, and four invertebrates, as food for fish (Table 2), were deliberately introduced (Zenkevich 1957; 1963).

History of introductions. In the late 1800's and early 1900's attempts to introduce an oyster and the mussel, *Mytilus,* failed. In 1930 and 1931, a flounder, *Pleuronectes flesus,* was introduced. It has never been abundant, though occasional specimens are still caught. One other flounder, *Rhombus maximus,* was planted but did not succeed.

In the early 1930's two species of mullet, *Mugil auratus* and *M. saliens,* from the Sea of Azov were successfully introduced. They are found primarily in the southern part of the Caspian Sea where they support a substantial commercial fishery.

Two shrimp species, *Leander squilla* and *L. rectirostris,* were stocked in the 1920's and now thrive in the Caspian Sea. A mullid, *Mullis barbatus,* and an engraulid, *Engraulis encrasiocholus,* from the Sea of Azov failed to become established.

TABLE 2

Useful animals introduced into Caspian Sea [Salinity 12 to 13 ppt[1]]

Common name	Scientific name	Year
Fishes		
Mullet	*Mugil auratus*[2]	1930's
Mullet	*Mugil saliens*[2]	1930's
Flounder	*Pleuronectes flesus*[3]	1930, 1931
Mosquitofish	*Gambusia affinis*	1920's
Invertebrates		
Shrimp	*Leander squilla*	1920's
Shrimp	*Leander rectirostris*	1920's
Worm	*Nereis diversicolor*	1939, 1940
Mollusk	*Syndesmya ovata*	1939, 1940

[1] From Zenkevich (1957; 1963).
[2] Important in the commercial fishery.
[3] Minor part of the commercial fishery.

Introduction of *Nereis diversicolor* was a well conceived move to improve the food base for fishes. It was notably successful in providing an additional, and more nutritious, source of food for sturgeons. In the period 1939 to 1941, about 65,000 *Nereis* were planted in the Caspian Sea. By 1944, they were a principal food organism for sturgeons in the northern part of the Caspian Sea. By 1948, the worm was present in an area of 12,000 square miles (30,000 square kilometers). Their weight was as much as 86 gm per m^2 of bottom. *Nereis* is an especially valuable adjunct to the fauna because of its behavior. The worm lives in soft silt at the bottom and can survive short periods without oxygen. After it lives for 1 year in the silt it becomes free swimming, spawns and dies, at which time it is readily available as fish food. In this way, it recycles material otherwise lost in the bottom.

Accidental introductions. The construction of the Volga-Don Canal in 1952 led to the accidental introduction of numerous species, many of them harmful fouling organisms carried by ships. The canal provided a route for migration between the Baltic Sea and the Caspian Sea. Zenkevich (1963) held that it will probably lead to the reconstruction of the Caspian fauna. He listed 11 invertebrates (including 2 barnacles and the harmful crab, *Rhithropanopeus harrisii*) and 10 species of algae that have entered the Caspian through the canal. In the same way, five Caspian species have emigrated to the Baltic Sea. One of them, *Cordylophora caspia*, is becoming cosmopolitan and has already been reported occurring in large seaports in North and South America, Australia, New Zealand and China.

Another bivalve mollusk, *Mytilaster* sp., was introduced into the Caspian Sea accidentally, probably carried on the hulls of small vessels from the Black Sea in 1917 to 1920, and by the 1940's was the most abundant benthic species (90% of the benthos). An accidentally introduced diatom, *Rhizosolenia calcar-avis*, is the dominant species in the phytoplankton.

ARAL SEA

The Aral Sea has an area of about 24,900 square miles (64,490 square kilometers), a maximum depth of 223 feet (68 m) and a salinity of 10 ppt (Zenkevich 1957). The ionic composition of the water clearly indicates a freshwater origin, and its fish fauna is entirely freshwater. There are, however, a few marine elements in the flora and fauna.

The introduction of Baltic herring, *Clupea harengus membras* L., into the Aral Sea (Bikov 1960) is another example of the introduction of species into a relict sea. Prior to the introduction of Baltic herring no Clupeids were present in the lake.

Zenkevich (1963) mentioned, besides the Baltic herring, the attempted introduction of five additional species of fish; two bullheads (gobies), *Pomatoschistus caucasicus* and *Gobius melanostomus*, a sturgeon, *Acipenser stellatus*, and mullets, *Mugil auratus* and *M. saliens*. It is not clear that any of these fish have been successful. A fish, *Atherina mochon*, was accidentally introduced. In 1958, attempts were made to introduce two species of *Leander*, from 1954 to 1956, attempts were made to establish three mysids, *Mesomysis kowalewski*, *Mesomysis intermedia*, and *Paramysis baeii*.

SALTERNS AND INLAND BRINES: SALTON SEA AND LAKE QUARUN

SALTON SEA

The Salton Sea has an area of about 340 square miles (880 square kilometers) and a maximum depth of about 40 feet (12.2 m). Like the Caspian Sea, the Salton Sea is below sea level (235 feet or 71.6 m). Salinity in 1964 was 33 ppt. It is located in a region with a severe desert climate. Failure of an irrigation headworks on the Colorado River in 1905 led to the accidental diversion of the entire river flow into the Salton Sink, creating the modern Salton Sea (Carpelan 1961). Not until 1907 was the river diverted back into its original channel. By that time a fresh-water lake covering 410 square miles (1,100 square kilometers)·had been formed. In the late 1930's, construction of Hoover Dam allowed extensive irrigation of the region around the Salton Sea and the sea has since been used as a drainage basin for waters from irrigated lands. Sufficient water must be applied to these fields to carry salts down through the soils and counteract the effects of evaporation.

Initial rapid increase in the salinity was caused by solution of minerals from the bed of the lake (Hely *et al.* 1966), but the later increase in salinity of the Salton Sea is traceable to the inflow of irrigation water (salinity about 2 ppt) balanced by evaporation rates of about 6 feet (1.8 m) per year. The minerals in this irrigation water are of course left behind and cause an increase in the salinity of the lake. Estimates of future salinity depend on assumed values for inflow, but Hely *et al.* (1966) state that by 1980 it could be as low as 31 ppt or as high as 120 ppt.

Faunal history. In 1916, a freshwater fauna, consisting of (in order of abundance) carp, *Cyprinus carpio,* striped mullet, *Mugil cephalus,* humpback sucker, *Xyrauchen texanus,* bonytail, *Gila robusta,* rainbow trout, *Salmo gairdneri* ("fairly common"), and the desert pupfish, *Cyprinodon macularius,* was present in springs around the periphery (Evermann 1916). According to Hely *et al.* (1966) the salinity in 1916 was 16 ppt.

By 1929, the salinity had increased to 33 ppt and the carp and bonytail had disappeared, the mullet had become scarce, but trout were still present near the inlets (Coleman 1929).

As recently as 1952, the Salton Sea contained only four species of fish. Three were small species not useful to man and limited, for the most part, to shallow waters near shore. The fourth species, the mullet, *Mugil cephalus,* is not particularly prized as a food fish in the United States and is not normally considered a sport or game fish, although a small commercial fishery for it existed in the Salton Sea at one time (Hendricks 1961). The mullet has never spawned in the Salton Sea and depends on natural recruitment from the Gulf of California. Juvenile mullet enter the Colorado River from the Gulf of California and pass through irrigation laterals to the Salton Sea.

History of introductions. After a survey in the late 1920's (Coleman 1929), biologists of the California Department of Fish and Game began attempts to introduce desirable species of fish into the Salton Sea. Because of the very limited fauna in the Salton Sea, it was essential to introduce invertebrates as food for fish (Table 3). The successful introduction of *Neanthes succinea* in 1930 was the key to the success of the whole enterprise.

Attempts have been made to introduce a total of 29 invertebrates. Most were chosen for their potential value in enlarging the food base for fish. Only 2 of the 29 species have successfully become established (Carpelan 1961); the pile worm, *Neanthes succinea,* and the amphipod, *Carinogammarus mucronatus.* The amphipod was introduced incidentally during attempts in 1957 to introduce, from Texas, the shoal grass, *Diplanthera wrightii,* as a food for water fowl. The plant was not successful, but the amphipod associate of the grass was established and is utilized as food by fish. A barnacle, *Balanus amphitrite* Darwin, was probably brought in by seaplanes in the 1940's.

Introductions of 35 species of fish were attempted during the period 1929–56. In all, four of these species have become established in the Salton Sea (Douglas 1953; Walker *et al.* 1961).

The mudsucker, *Gillichthys mirabilis,* was the first successfully introduced fish in 1930.

The bairdiella, *Bairdiella icistius,* was introduced in very small numbers (67 individuals) during 1950 and 1951. Its spawning in 1952 marked the first occupation of Salton Sea by a fauna destined to support a sport fishery. Although bairdiella is not a desirable sport fish, because of its small size (11 inches maximum total length), it did fulfill an essential role in the food chain. Bairdiella

TABLE 3

Present fish and invertebrate fauna of the Salton Sea and its origin [Salinity 33 ppt[1]]

Common name	Scientific name	Introduced by man	Date	Self-introduced through irrigation canals	Native fauna
Fishes					
Orangemouth corvina	*Cynoscion xanthulus*	X	1950–56	.	.
Sargo	*Anisotremus davidsoni*	X	1951	.	.
Bairdiella	*Bairdiella icistius*	X	1950–51	.	.
Mudsucker	*Gillichthys mirabilis*	X	1930	.	.
Striped mullet	*Mugil cephalus*	.	......	X	.
Threadfin shad	*Dorosoma petenense*	.	......	X[2]	.
Mosquitofish	*Gambusia affinis*	.	......	.	X[3]
Desert pupfish	*Cyprinodon macularius*	.	......	.	X
Invertebrates					
Pile worm	*Neanthes succinea*	X	1930	.	.
Amphipod	*Carinogammarus mucronatum*	X	1957	.	.
Barnacle	*Balanus amphitrite*	X	1944?	.	.
Copepod	*Cyclops dimorphus*	.	......	.	X
Nematode	*Spilophorella* sp.	.	......	.	X
Rotifer	*Brachionus plicatilis*	.	......	.	X
Protozoa	Numerous species	.	......	.	X

[1] From Carpelan and Linsley (1961) and Walker, Whitney and Barlow (1961).
[2] Introduced by man into Colorado River (Kimsey 1954), from whence it spread to the Salton Sea.
[3] Introduced by man into area prior to formation of Salton Sea.

feed on *Neanthes* in the deeper waters of the Salton Sea, and thus form a link between *Neanthes* and larger predators.

The success of the larger predator, the orangemouth corvina, *Cynoscion xanthulus*, was dependent on the success of the bairdiella and the two will be discussed together. The corvina spawned in 1952, but less successfully than the bairdiella (Walker *et al.* 1961). The corvina was derived from the introduction of about 200 fish in 1950 and 1951. Although the success of bairdiella in the Salton Sea was sudden and represented a "population explosion," the corvina population developed slowly (Whitney 1961) because the juvenile corvina had to compete for food with the bairdiella. Young-of-the-year of both species fed on copepods, barnacle nauplii and, later, *Neanthes* (Quast 1961; Whitney 1961). The 1953 year class of overpopulated bairdiella was stunted, and succeeding year classes were suppressed because of competition with this year class. Each intervening year class until 1957 was somewhat more successful than the preceding one as mortality gradually reduced the numbers of the 1953 fish. The success of the corvina year classes followed the same course. Once the corvina of a particular year class reached a size where they could begin to feed on bairdiella, their growth rate increased suddenly. Numbers of young-of-the-year bairdiella were sufficient by 1955, that the corvina could shift from feeding on *Neanthes* to feeding on fish at an early age. Large numbers of corvina of this year class were found, and succeeding year classes were even more abundant. The corvina was well established in Salton Sea after 1955 and introductions were terminated in 1956.

Another species which was slow to become abundant was the sargo, *Anisotremus davidsoni*. Fifty-seven individuals were stocked in 1951 by the California Department of Fish and Game. They were rare in 1954–57, when only three specimens were caught in samples of more than 30,000 fish, but by 1960 the species was sufficiently abundant to support a sport fishery.

The threadfin shad, *Dorosoma petenense*, was accidentally introduced into the Salton Sea. By September 1955 it had spread into the Salton Sea through irrigation laterals, after having been introduced in the Colorado River in November 1954 (Kimsey 1954). It does not spawn successfully in the lake, but is recruited from the freshwater inlets (Hendricks 1961).

The corvina, sargo, and bairdiella now support a sport fishery worth $3,000,000 per year (Pomeroy *et al.* 1965). Estimated catch in 1965 was 540,000 corvina, 145,000 sargo and 148,000 bairdiella (unpublished estimates, courtesy R. Hulquist and J. St. Amant, California Department of Fish and Game). The catch and effort are on the increase.

Future of the Salton Sea fishery. The present fish fauna consists of eight species, including the four species present before 1952 (only one of which, *Gillichthys*, had been intentionally introduced by man) and the four recently introduced species (the corvina, bairdiella, sargo and threadfin shad).

The very simple combination of species in the Salton Sea uses the available energy in a food chain leading to the corvina. Walker *et al.* (1961) recommended that no further introductions of fishes be attempted because of the demonstrated success of the present combination of species and because of the danger of introducing undesirable species. On the other hand, the invertebrate food chain seems weak because *Neanthes* is the single food source consisting of individuals large enough to support the fish serving as the principal prey for corvina. The introduction of mysids and amphipods was recommended.

The gradually increasing salinity of the Salton Sea, unless checked, will someday eliminate the fish population. Walker *et al.* (1961) estimated that the sport fishery might terminate after the decade 1980–90. They assumed a maximum salinity tolerance of about 45 ppt and that the salinity would continue to increase at 0.4 ppt per year. Studies of salinity tolerances for related fishes in the Texas Laguna Madre (Simmons 1957) suggest that they might have underestimated the salinity tolerance of these fishes by 10 or even 20 ppt, although the more important species in the Salton Sea have not been studied. Sport fishery and other recreational uses of the Salton Sea have stimulated recent studies of the factors affecting the salinity of the Salton Sea and led to suggestions for controlling the salinity by removal of water either by pumping or by constructing a system of dikes to limit the surface area and reduce the quantity of water evaporated (Hely *et al.* 1966). Eventually it will be necessary to regulate the salinity of the Salton Sea, if the sport fishery is to be maintained. The time is approaching when this step can be economically justified.

LAKE QUARUN

Introduction of fish species into an inland saline lake in a climate similar to that in the region of the Salton Sea was reported by El Zarka (1963). Lake Quarun in Egypt is a small lake of 53,000 acres (22,000 hectares) and has a maximum depth of 26 feet (8 m). Average noon air temperatures range from 17.6 C in January to 34.2 C in July. Evaporation from the surface of the lake amounts to about 6 feet (1.8 m) per year. The use of the lake as a storage basin for overflow from the Nile, the water from which was retained for use during the dry season, originated in prehistoric times and continued until 1920. Subsequent to the construction, in the 1920's, of the Fayoum irrigation system upstream from Lake Quarun, the lake has served as a drainage basin for irrigated lands. Its salinity increased from 11.6 ppt in 1920 to 29.4 ppt in the 1960's. The original freshwater fish fauna of six principal species was eliminated by 1936, except for the presently dominant *Tilapia zilli.*

In 1928–35, mullet, *Mugil cephalus* and *M. capito,* were successfully introduced (Table 4) from the Mediterranean (Faouzi 1936). They have supported a sizable commercial fishery. By 1935, the commercial catch amounted to 377 tons

TABLE 4

Fishes introduced into Lake Quarun, Egypt [salinity 29.4 ppt[1]]

Common name	Scientific name	Year
Striped mullet	*Mugil cephalus*	1928–35
Mullet	*Mugil capito*	1928–35
Sole	*Solea vulgaris*	1938–48

[1] From El Zarka (1963).

(341,726 kilograms) per year. They are still an important part of the commercial catch—18% of the total lake catch.

Fishing has been further augmented by the introduction of a Mediterranean sole, *Solea vulgaris*. A total of 1,603 individuals were stocked over a period of 10 years and commercial fishing for the species began in 1943. In 1960, the most recent year reported by El Zarka (1963), the catch was 151 tons (136,849 kilograms). The largest catch of 299 tons (271,035 kilograms) was recorded in 1959. The catch of sole represents 37% of all species from the lake, and exceeds the catch of that species from the Mediterranean.

CARBONATE AND SULFATE WATERS: SALINE LAKES OF SASKATCHEWAN

This section should perhaps be a subsection under the previous category since the two are obviously related. Clarke (1911) reviewed the history of these waters, explaining it as being the result of differential solubility of naturally occurring ions. Carbonate and sulfate waters became sodium and chloride waters. Moore (1952) reported the precipitation of carbonates and sulfates with increasing concentration of ions in the saline lakes of Saskatchewan.

These Saskatchewan lakes are glacial remnants without outlets. The salinity varies from lake to lake, from less than 0.3 to 120 ppt (Table 5), and increases annually at a rate depending on the size of the lake and its inflow.

None of these lakes is of the chloride type. The dominant ions are bicarbonate and sulfate. They are similar in many respects to lakes of the Siberian steppes and to Devils Lake, North Dakota.

The potential of the saline lakes of Saskatchewan as producers of commercial fish was apparently first recognized by Huntsman (1922) and later documented by Rawson and Moore (1944). Huntsman's experiments in the introduction of cisco, *Coregonus artedii*, and whitefish, *C. clupeaformis*, into the Quill Lakes in the years 1924–27 were successful. Both species survived and supported a commercial fishery. In 1920, the salinity was 10.8 ppt in Little Quill Lake and 16.5 ppt in Big Quill Lake; but by 1944, the salinities had reached 20 ppt and 29 ppt, respectively, and the fish were no longer present (Rawson and Moore 1944). In

TABLE 5

Number of asline lakes in Saskatchewan with the list range of salinities[1]

	Number of lakes	Salinity (ppt)
	1	120
	7	10–30
	19	1–10
	22	0.3–1
	11	< 0.3
Total	60	. . .

---

[1] From Rawson and Moore (1944).

the intervening years, up to the early 1940's, five species of fish were successfully introduced into saline lakes (Table 6).

One of the lakes, 27-square-mile (70 square kilometers) Redberry Lake, had a salinity of 15 ppt in 1944 (Rawson 1946). The existing fish population consisted of fathead minnows, *Pimephales promelas,* and two species of stickleback, *Eucalia inconstans* and *Pungitius pungitius.* In the period 1940–44, the lake was stocked with 500,000 to 1,000,000 whitefish, *Coregonus clupeaformis,* fry per year. The introduction was successful and by 1946 the commercial catch of whitefish from Redberry Lake amounted to 20 tons (18,000 kilograms) per year. Rawson found that the rate of growth of whitefish was twice that in neighboring lakes, but he found no evidence of natural spawning. The whitefish were of exceptional quality, particularly because of the absence of parasites in their flesh, a feature which lowers their quality in many other waters.

THE FUTURE

Animals which have been most successfully introduced or cultured in saline waters, and therefore, can be considered to have a high probability of success in future attempts, are as follows:

Fishes
  Family
    Mugilidae—*Mugil cephalus* introduced into Lake Quarun, self introduced in Salton Sea, and cultured in saline ponds in Europe and Asia. *M. capito* introduced into Lake Quarun and Caspian Sea and cultured in saline ponds in Europe. *M. auratus* also introduced into Caspian Sea and cultured in saline ponds in Europe. Other species of *Mugil* cultured in saline ponds in Europe and Asia.
    Elopidae— *Elops hawaiiensis* and *Megalops cyprinoides* cultured in saline ponds in Asia. *Elops affinis* self introduced for a short time in Salton Sea. (Dill and Woodhull 1942)
    Pleuronectidae—*Pleuronectes flesus* introduced into Caspian Sea and cultured in saline ponds in Europe.
    Soleidae—*Solea vulgaris* introduced into Lake Quarun.
    Clupeidae—*Clupea harengus* introduced into Aral Sea. *Dorosoma petenense* introduced into Salton Sea.

Table 6

Introduction of fish into saline lakes of Saskatchewan[1]

Common name	Lakes attempted	Lakes successful[2]
Walleye	15	12
Yellow perch	12	7
Lake whitefish	6	4
Northern pike	6	2
Cisco	3	2
Largemouth bass	13	0

[1] From Rawson and Moore (1944).
[2] Successful was defined as surviving in considerable numbers for at least 5 years.

Sciaenidae—*Cynoscion xanthulus* and *Bairdiella icistius* introduced into Salton Sea.
Chanidae—*Chanos chanos* cultured in saline ponds in Asia.
Cichlidae—*Tilapia mossambica* cultured in saline ponds in Asia and introduced into stream tributary to Salton Sea.
Coregonidae—*Coregonus clupeaformis* introduced into three saline lakes in Saskatchewan.

Invertebrates
  Family
    Nereidae—*Neanthes succinea* introduced into Salton Sea. *Nereis diversicolor* into Caspian Sea.
    Penaeidae—Various species of *Penaeus* cultured in saline ponds in Asia.
    Palaemonidae—*Leander squilla* and *L. rectirostris* introduced into Caspian Sea.

Culture of useful animals in ponds could be of greatest utility in mediating any harmful effects of effluents from desalination plants. Possibly, this culture could even be carried out in evaporation ponds and the salt could be marketed along with useful animals such as brine shrimp that would certainly appear, and other animals that might be introduced into such ponds at times when the salinities were suitable. The value of some shrimps or of bait fishes, *e.g.*, the mudsucker, *Gillichthys* spp., in the United States exceeds the value of food fishes that might be raised in such ponds. It would, therefore, be logical to concentrate on their culture to maximize the economic returns. The probable economic returns would no doubt justify some control of salinities in the ponds at less than lethal levels for the fish or shrimp (less than about 70 ppt for *Gillichthys*, Barlow 1961).

The beneficial effects of fertilizing salt-water ponds have been amply demonstrated ,Gross *et al.* 1944; Gross *et al.* 1946; Raymont 1947a; 1947b; 1948; 1949; Marshall and Orr 1948; Pratt 1949; Chimits 1955). Imaginative design of pond systems accordingly should take into consideration the utilization of sewage effluents, both for control of salinity and fertility. Ponds could also be benefited by the heated effluents from coastal electric power stations which use the water for condenser cooling. Shelbourne (1964) suggested that ponds for culture of marine fish could be placed near the outflow from coastal atomic power stations to take advantage of the elevated water temperature. The elevated water temperature would facilitate the desalination processes, and could make possible the culture in the continental United States of fish such as the milkfish which require and thrive in higher temperatures, or it could make possible the culture in northern regions of the United States of desirable fishes such as the Gulf of Mexico sciaenids, which are known to thrive in high temperature-high salinity habitats.

But future work in the introduction of commercially important species should not be limited to areas affected by effluents from desalination plants. Hypersaline and mineral waters occur naturally throughout the world. The success recorded in the few attempts made to introduce commercial species into such waters, and the field studies which have demonstrated the fact that species useful to man can survive in such waters, ought to stimulate further work in this field.

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